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EFFECTS OF X-RAYS ON HATCHABILITY AND ON CHROMOSOMES OF HABROBRACON EGGS TREATED IN FIRST MEIOTIC PROPHASE AND METAPHASE[1]

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IN 1904 Bergonié and Tribondeau found that in rat testes integumental cells were highly resistant while dividing cells were very sensitive to irradiation. In 1906 they concluded that x-rays affect the cell in direct proportion to (1) its reproductive activity, (2) earliness of its position in the series of divisions before maturity and (3) its degree of generalization in structure and function. In this publication they suggested that sensitivity of tumors to irradiation is due to the mitotic activity of their cells. In the same year Kraus and Ziegler found the time of organization of the equatorial plate the most sensitive stage and stated that it is less the kind of cell than its stage at time of treatment which determines sensitivity. Holthusen in 1921, after a thorough study of early cleavage in irradiated Ascaris eggs, agreed with the first and third conclusions of Bergonié and Tribondeau but substituted for the second the conclusion of Kraus and Ziegler. He observed that sensitivity is strikingly high when chromosomes begin to appear and

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is at its maximum at the construction of the metaphase plate.

Many investigators have studied the effect of x-rays on many kinds of cells since this earlier work and the majority, using diverse criteria, has favored the prophase as the most sensitive stage. In many cases, if not most, the metaphase has not been adequately studied either because of the difficulty involved in "catching" the cells in this relatively brief stage or of complication due to "physiological" effects, clumping and stickness of metaphase and anaphase chromosomes after irradiation.

A summary of more recent studies of irradiated oocytes is presented here. In the mature Drosophila female most of the older oocytes are in prophase stages in preparation for the first meiotic division and, with an occasional exception, none is further advanced than metaphase I. Patterson and Muller (1930) announced that both point mutations and chromosomal rearrangements were produced by raying either sex in Drosophila but in considerably greater abundance in the sperm than in the oocytes with a given dose and that the ratio of rearrangements to point mutations was higher in treated sperm than in similarly treated female germ cells. They postulated a difference connected with the sex of the cell as a factor in sensitivity. Patterson, Brewster and Winchester (1932) found that eggs retained in the ovaries of Drosophila became more sensitive to irradiation and concluded that the nearer eggs are to maturity, the more susceptible they are to x-rays with regard to production of chromosome breaks. This susceptibility, they suggested, is probably related to the condition of the chromatin.

Oliver (1934) showed that, when young Drosophila females are treated, sex-linked lethals, translocation and crossingover irregularities occur less frequently than with male treatment but that visible variations occur in equal numbers in both sexes. Shapiro and Volkova (1938) state that "Comparison of natural and induced mutation processes showed that latter occur more often in males than in females whereas former display

no such difference, probably due to fact that x-rays induced more large chromosome aberrations in spermatoza." Glass (1939) found no translocations and fewer inversions in larvae from treated females than in larvae from similarly treated males in Drosophila while Sonnenblick (1940) showed for this species that "especially susceptible to the radiations are the most mature oocytes."

Schultz (1933) reported similar sex differences in response to x-rays in *Drosophila pseudoobscura.*

In 1938 the author stated that unlaid eggs of Habrobracon are most sensitive to irradiation in late metaphase I, almost equally so in latest prophase I (diakinesis) and least so in prophase stages before diakinesis. Adult (haploid male) survival from unfertilized eggs laid by treated females was used as the criterion. Later (1940) the data were presented in detail.

Metz and Boche (1939) observed that irradiation of adult females of Sciara gave rise to no gross chromosomal changes in F_1 larvae in contrast to irradiation of adult males and it was demonstrated by Metz and Bozeman (1940) that these eggs were in mid-prophase when treated. Reynolds (1941), by treating Sciara females restrained from ovipositing until eggs have reached stages later than mid-prophase, has found "that oocytes increase in sensitivity as they pass through the late prophase stage of meiosis. . . . It is interesting to note that the metaphase is the most sensitive stage and that here even with comparatively low dosages a high frequency of breaks occurs." Reynolds concluded that difference in sensitivity is not a matter of sex but of chromosome condition at time of treatment, thereby agreeing with Kraus and Ziegler (1906). From new data (unpublished) Reynolds finds that early anaphase may be even more sensitive than metaphase.

The author, in 1941, announced that hatchability percentages of unfertilized Habrobracon eggs x-rayed in late prophase I with doses up to 400 r are significantly higher than those of controls and that lethal dose is about 35,000 r. Eggs treated in late metaphase I are sensitive to 50 r

and lethal dose is about 1,250 r. There is no evidence of recovery in this stage. The dose-hatchability curves of the two stages differ, that of metaphase I showing a linear relationship to dose. In 1942 evidence against "physiological effect" as an important factor for metaphase I sensitivity was presented as well as preliminary cytological observations.

Bozeman (1942) reports that viable chromosome alterations are first detectable in Sciara larvae developing from eggs treated when chromosomes begin to shorten and move to metaphase I plate. Sensitivity increases through metaphase I and early anaphase I, then decreases during the arrested stage (anaphase). Just before fertilization sensitivity increases. A high frequency of intrachromosomal rearrangements and an unequal distribution of types of aberrations when different stages of meiosis are treated are reported and the latter fact is used as an indication that rearrangement is not delayed until fertilization.

MATERIAL AND METHODS

Two criteria for judging injury are used in the present study. One of these, hatchability, gives degree of lethal effect, both dominant and recessive, and is a very accurate measurement in Habrobracon where unfertilized haploid eggs are capable of development, where every egg can be accounted for, where the ideal environmental conditions are known and easily controlled and where there is available a stable wild-type stock consistently giving 96 per cent. hatchability or higher. The second criterion, chromosome changes in successive stages of the treated oocytes, is less satisfactory because Habrobracon chromosomes are too small (1 μ in diameter) and too numerous ($n = 10$) for very careful analysis. Giant salivary chromosomes are lacking. Enough has been learned from cytological observation, however, to make this method of some value in connection with hatchability data. The chromosome aberrations observed are neces-

sarily gross ones and undoubtedly represent lethal effects in most cases, effects which escape observation in salivary chromosome studies.

Unmated mature Habrobracon females, when well fed on host caterpillars and then restrained from ovipositing for several hours by removal from host, store in each of their four egg sacs from two to five fully mature eggs which may be retained for at least thirty-six hours without injury. After a much longer period of storage they undergo resorption. They are all in late metaphase I with spindle attachment regions separated and about half way to the poles. The ends of the dyads are still in contact so that the chromosomes appear to be under tension. Occasionally, an egg in late diakinesis or in early metaphase I can be seen entering an egg sac. Successively younger prophase oocytes occupy the ovarioles anteriorly. Synapsis occurs as soon as youngest oocytes can be distinguished from oogonia. An ovariole is represented semidiagramatically in Fig. 1.

A count of eggs in dissected egg sacs of thirty females (1940) deprived of food for twelve hours gave the mean number in late metaphase I as 13.2 per female, of these plus those in early metaphase I and diakinesis as 14.3. In this earlier study eggs of treated (2,500 r) unmated females were collected in order of laying. The first few eggs laid failed to hatch and mean number of those laid before first to hatch was 13.1 per female, of eggs failing to produce adults before first adult was 14.5. It was concluded that all eggs in late metaphase had failed to hatch, that those in early metaphase and diakinesis had hatched but died as larvae. A pause in laying takes place after non-hatching eggs have been laid. At 30° C. this occurs between the sixth and seventh hour after the beginning of oviposition.

About sixteen oocytes which are unquestionably beyond the synaptic stage can be seen in each ovariole between the egg sac and the newly differentiated oocytes. With the technique used in this work control females lay

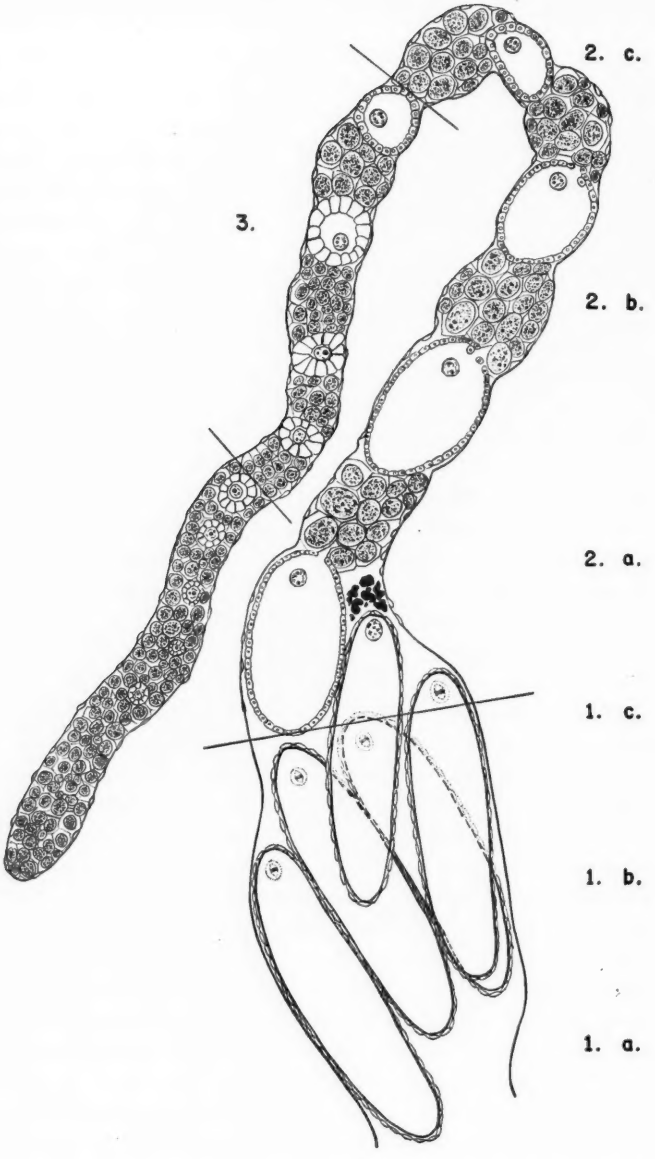

FIG. 1. A single ovariole (semidiagrammatic) showing regions used in classification of treated eggs. 1a, b and c include eggs in metaphase I stored in egg sac; 2a, b and c include eggs in metaphase I, late and mid prophase I; 3 includes eggs in early prophase I. All are post-synaptic. ×85.

an average of 11.3 (5,099/453) eggs per day which means that, at this rate, seven and one-half days would be required to lay the maximum number of metaphase I plus prophase I eggs present at any given time, that is, about eighty-five eggs. Since, in experiments, eggs are collected for one, two or three days only, they are without doubt post-synaptic when treated. Maximum number laid by treated females was twenty-one for day 1, forty-six for days 1 and 2 and 53 for days 1, 2 and 3. For those which remain to be laid or to disintegrate (if very young) there is, therefore, a margin of thirty-two or more before the post-synaptic number is exhausted.

The technique for experiments herein reported is as follows. Well-fed unmated females, all from the same vigorous wild-type stock, are deprived of food for twelve hours during which time, (as also throughout the experiments, except while being irradiated) they are kept at 30° C. unless otherwise stated. They are set immediately after treatment, still unmated unless otherwise indicated, on host caterpillars and are transferred to fresh hosts every two hours for six hours. Caterpillars, previously paralyzed by "nurse" wasps (females sterilized by x-rays), are used for this. The mean number of eggs per treated female during this six hour period has been 10.3 (13,835 eggs, 1,343 females), a number smaller than mean number of those observed to be in metaphase I in the egg sacs. At 1,800 r and all higher doses no egg laid during these first six hours after treatment has hatched among 8,991 studied, a clear indication that they were all in metaphase I when treated. Eggs are counted when laid and records are taken of their condition after a thirty-six hour incubation period. In later experiments hatchability has been substituted for adult survival since it is not affected by such environmental conditions as size of caterpillars, disease, etc.

When eggs which have been irradiated in prophase I are desired, females which have been laying for six hours as described above are removed from the host, placed in

the incubator over night and then put on new hosts the day after treatment. During the first two hours of this second day of collecting, a small but variable number of eggs which were in metaphase I and diakinesis when treated are laid along with some which were in late prophase I. This variation is indicated by the great differences in hatchability of this group at any given dose. Females were transferred every two hours for six hours as on previous day. The process is repeated if eggs are to be collected on the second day after treatment. Treated females become practically sterile after this time, except when exposed to light doses.

Reference to Fig. 1 will help to make this method clear. For convenience in discussion all eggs laid during the day of treatment are said to belong to group 1a, b and c; those laid on the day after treatment to group 2a, b and c; those on the second day after treatment to group 3. All eggs in group 1 were in late metaphase I when treated, those in 2a in metaphase I, diakinesis or late prophase I, those in groups 2b and 2c probably in mid-prophase I and group 3 in early prophase I.

Two large control groups were studied during the course of the experiments. The first, in March, 1941, gave a hatchability percentage of 91.1 ± 0.6 (1,864/2,045), the second, in March, 1942, 96.6 ± 0.4 (1,522/1,575). This increase was the result of selection for high hatchability. In some experiments eggs of females were used as controls for eggs laid by the same females after treatment. This is legitimate since age of mother has no effect on hatchability for periods greatly in excess of any used. Standard errors are used throughout the paper.

Treatments were given at the Department of Radiology of the University of Pennsylvania, the Marine Biological Laboratory at Woods Hole and the American Oncologic Hospital of Philadelphia. At the University of Pennsylvania conditions of treatment were 200 kv., 20 ma., 15 cm. target distance, $\frac{1}{2}$ mm. copper plus 1 mm. aluminum filter, 500 r per minute. At Woods Hole the dual-tube self-

rectifying outfit with a simultaneous cross-firing technique was used. The secondary voltage was 182 kv. and the tube current on each tube was 25 ma. The heavy glass of the tube walls and 5 mm. of bakelite of the tube shields gave the filtering value of 0.2 mm. copper shield. The output intensity was 560 r or 5,600 r per minute, depending on distance, 30 cm. for former, 9.5 for latter. At the American Oncologic Hospital conditions were 140 or 200 kv., 25 ma., and an inherent filter with the value of 6.0 mm. aluminum shield for 200 kv. and 3.8 for 140 kv. Output intensity varied from 220 to 1,350 r per minute. Time was the variable except where otherwise indicated. All high treatments for prophase studies were given at the Marine Biological Laboratory.

Experiments

Eggs Irradiated in Late Metaphase I (1a, b, c). Dose-survival curve for Experiment 1 and dose-hatchability curves for Experiments 2–5, which concern eggs laid during six hours of the day of treatment, are plotted in Fig. 2. Data are given in Tables 1–3. Percentages are corrected since control survival and hatchability were

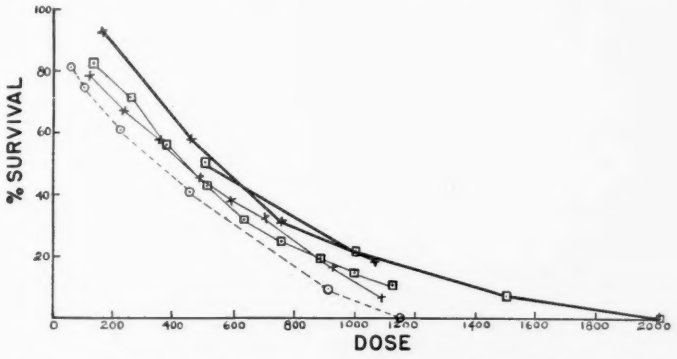

Fig. 2. The relation between x-ray dose in r units and (1) corrected adult survival percentages (dotted line) and (2) corrected hatchability percentages for treatments administered (a) at the University of Pennsylvania Hospital (light solid lines) and (b) at the American Oncologic Hospital (heavy solid lines). Eggs were treated in late metaphase I.

TABLE 1

CORRECTED ADULT SURVIVAL PERCENTAGES FOR EGGS TREATED IN LATE METAPHASE I; UNIVERSITY HOSPITAL. EXPERIMENT 1

Dose in r units	Number of eggs	Survival percentage
50	64	81.1
103	81	73.7
225	96	60.9
455	78	41.5
910	55	9.4
1,150	113	0.0

lower in earlier work. In subsequent figures and tables actual hatchability is given, control hatchability having become very high through selection. It is to be noted that the lower adult survival curve parallels the hatcha-

TABLE 2

CORRECTED HATCHABILITY PERCENTAGES FOR EGGS TREATED IN LATE METAPHASE I; UNIVERSITY HOSPITAL

Experiment 2			Experiment 3		
Dose in r units	Number of eggs	Hatchability percentage	Dose in r units	Number of eggs	Hatchability percentage
117	67	78.5	125	285	82.9 ± 2.8
233	55	66.9	250	305	71.3 ± 3.0
350	71	57.4	375	300	55.3 ± 3.2
484	94	45.5	500	260	42.7 ± 3.3
583	57	39.2	625	260	32.1 ± 3.1
700	120	32.1	750	434	25.6 ± 2.2
932	65	16.2	875	189	20.3 ± 3.1
1,089	39	6.1	1,000	136	14.5 ± 3.2
....	...	...	1,125	189	10.5 ± 2.3

bility curves of the other experiments. The space between them represents larval and pupal mortality. Numbers are too small to have statistical significance in Experiments 1 and 2. Standard errors are computed for the others.

Females used in Experiments 4 and 5 were x-rayed at

TABLE 3

CORRECTED HATCHABILITY PERCENTAGES FOR EGGS TREATED IN LATE METAPHASE I; ONCOLOGIC HOSPITAL

Experiment 4			Experiment 5		
Dose in r units	Number of eggs	Hatchability percentage	Dose in r units	Number of eggs	Hatchability percentage
159	262	92.1 ± 1.8	500	300	50.0 ± 3.0
450	465	57.3 ± 2.4	1,000	270	21.9 ± 2.6
750	697	31.1 ± 1.8	1,500	281	7.4 ± 1.6
1,060	307	18.2 ± 2.2	2,000	159	0.0

the Oncologic Hospital. A consistent difference exists in percentages of hatchability per given dose between their eggs and those of females irradiated at the University Hospital or at the Marine Laboratory. This may be due to a difference in method of calibration or in technique of handling.

Lethal dose for hatchability of eggs irradiated in late metaphase I is about 1,400 r for the University Hospital and Marine Laboratory treatments, about 2,000 r for the Oncologic Hospital. Fifty per cent. lethality is caused by 435 r and 500 r respectively.

Eggs Irradiated in Prophase I (2a, b, c, 3). An unexpected resistance to x-rays of late prophase I eggs was encountered in the first experiments reported (1938). Cytological study of the ovaries has demonstrated that all eggs classed as prophase have chromatin diffuse and but faintly stained by the Feulgen reaction, have completed synapsis and not yet reached diakinesis. This seems to justify the treatment of this group as a unit, at least for general conclusions.

Of nineteen experiments involving treatments of eggs in prophase I with doses ranging from 50 r to 750 r, seventeen had hatchability higher than controls, one the same as controls and one slightly lower. A summary of these nineteen experiments gives a hatchability of 95.2 ± 0.5 (1,569/1,647) with control hatchability of 91.1 ± 0.6 (1,864/2,045). The difference is 4.1 ± 0.8, five times its standard error. Further experiments are needed to check this suggestion, unorthodox at the moment, that small amounts of irradiation may stimulate development. Such stimulation, if it occurs, would have to be "physiological" in nature rather than chromosomal, perhaps comparable with that of freezing during diapause in certain insect eggs.

Uncorrected hatchability percentages of 2b plus 2c plotted against doses (Fig. 3) and data (Table 4) of Experiments 6, 7 and 8 illustrate the high resistance to x-rays of prophase I and the nature of the response. In

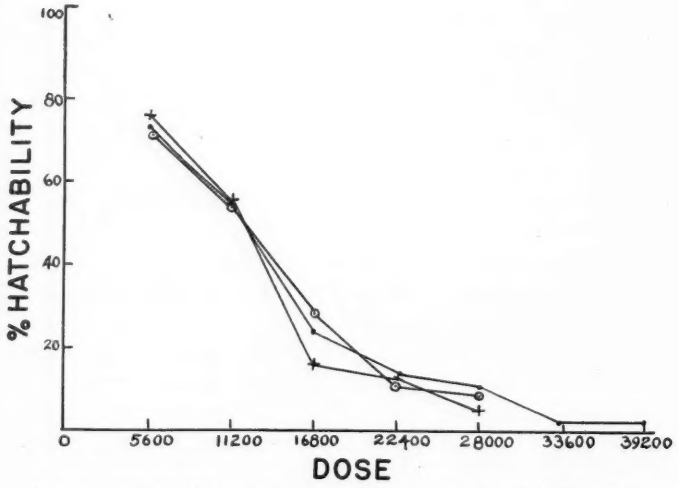

FIG. 3. Dose-hatchability curves of eggs treated in prophase I, three experiments.

Experiments 6 (1941) and 8 (1942) all females were irradiated at the same time for all doses and all were bred simultaneously. Experiment 7 (1942) was divided

TABLE 4

HATCHABILITY PERCENTAGES FOR EGGS TREATED IN PROPHASE I

Experiment	Dose in r units	2a		2b + 2c	
		Number of eggs	Hatchability percentage	Number of eggs	Hatchability percentage
6	5,600	51	7.8	98	75.7
	11,200	51	0.0	101	55.5
	16,800	52	0.0	101	16.0
	22,400	53	3.7	94	12.9
	28,000	48	0.0	119	4.9
7	5,600	173	43.9	393	72.5
	7,803	91	20.8	296	65.8
	11,200	227	40.5	259	56.7
	16,800	143	2.1	329	24.0
	22,400	242	3.3	264	13.6
	28,000	86	2.3	255	10.5
	33,600	224	0.4	295	2.0
	39,200	102	1.0	275	2.5
8	5,600	60	53.3	88	75.0
	11,200	25	12.0	111	58.5
	16,800	75	12.0	168	27.9
	22,400	65	9.2	137	11.6
	28,000	91	1.1	140	9.2
7 and 8	5,600	...	...	481	72.9 ± 2.0
	11,200	...	...	370	57.3 ± 2.6
	16,800	...	...	497	25.3 ± 1.9
	22,400	...	...	401	12.9 ± 1.6
	28,000	...	...	398	10.1 ± 1.5

into parts in order that larger numbers might be secured. Alternate doses were given at one time. After the highest treatments females appear unaffected and continue to live, sting hosts and feed although they lay almost no eggs beyond the second day after treatment since their ovaries have degenerated. 11,800 r produces 50 per cent. hatchability and 44,800 r approximates the lethal dose.

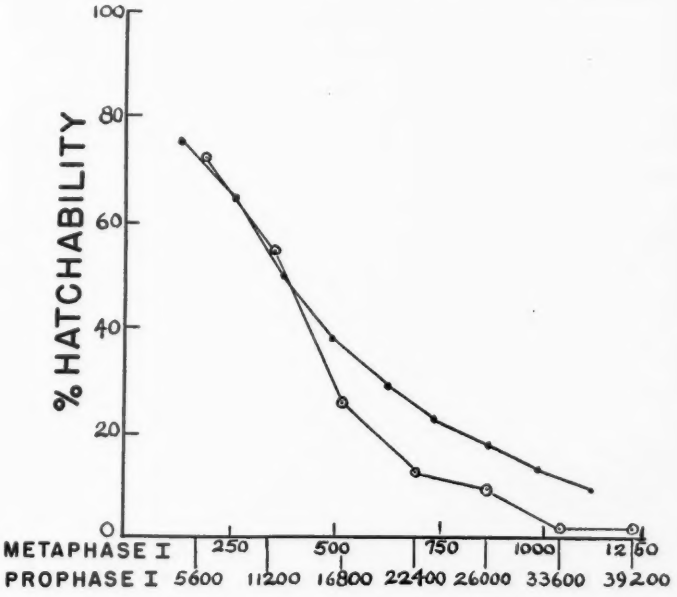

FIG. 4. Dose-hatchability curves of eggs treated in late metaphase I (●) and in prophase I (⊙) superimposed with points of 50 per cent. hatchability coinciding.

Although the chromosomes of the prophase I nuclei appear similar throughout the growth of the oocytes, hatchability data suggest that sensitivity to irradiation changes. A comparison has been made between hatchabilities of eggs laid during the second (2b) and third (2c) two hour periods of the day after treatment for each dose. 2a represents a mixture of eggs treated in late metaphase

I, diakinesis and latest prophase I and is, therefore, of no value in this study. Hatchability after treatment is higher in 2c (earlier) than in 2b (later) in eighteen of the twenty-one experiments. In five of the eight experiments where records were taken of hatchability on the second day after treatment (3, earliest prophase I studied) this group was found to have the lowest hatchability. In the other three experiments it fell between 2c and 2b in two and was higher than both in one.

These results indicate that hatchability of prophase I oocytes after irradiation decreases in the order of 2c, 2b and 3, *i.e.*, that earlier and later prophase I stages are more sensitive than the intermediate. This may be evidence for the dependence of sensitivity upon chromosome movement which would be greater in younger stages following synapsis and in older ones preparing for diakinesis.

Comparison of Eggs Treated in Late Metaphase I and in Prophase I. A comparison between the dose-hatchability curves of eggs treated in late metaphase I and those treated in prophase I is made in Fig. 4, where the curves are superimposed with points of 50 per cent. hatchability coinciding. Metaphase Experiment 3 is used here because of its large numbers and of the fact that it was done at the University of Pennsylvania so that hatchability is comparable with that following prophase treatments given at the Marine Laboratory. The two prophase I experiments (7 and 8) conducted within one week of each other are summarized for this comparison. Semilogarithmic representations of these curves are given in Fig. 5.

Eggs Irradiated and Fertilized by Untreated Spermatozoa. Are the lethal effects so far discussed dominant, recessive, or a mixture of both? If treated females are mated to untreated males two thirds of the eggs should be fertilized and, if any appreciable percentage of those that would fail to hatch as haploids could survive with the help of normal spermatozoa, it would be apparent

that some of the effects induced in haploids are recessive. A preliminary test of the effect of such matings on metaphase I was reported in the earlier paper (1940). Three females were irradiated with 2,500 r and then mated. No effect was observed, but this test was not conclusive since 2,500 r is higher than the lethal dose.

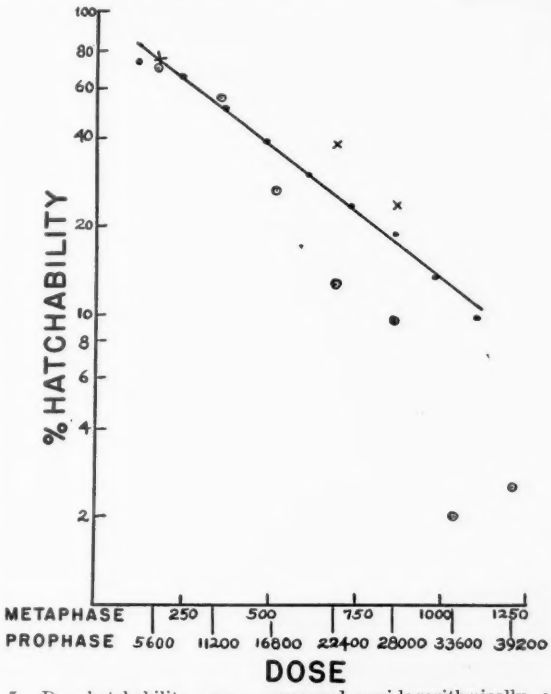

FIG. 5. Dose-hatchability curves, expressed semi-logarithmically, of eggs treated (1) in late metaphase I (●), (2) in prophase I, (a) continuous treatments (⊙) and (b) fractionated (×), superimposed with points of 50 per cent. hatchability coinciding.

In a recent experiment, summarized in Table 5, all the females of each dose group were treated and tested simultaneously. It will be seen from this summary that no apparent difference exists in hatchability between the mated and unmated groups in respect to eggs treated either in late metaphase I or in prophase I, at high and

low doses. Certainly it can be concluded that most, if not all, of the lethal effects induced by x-rays in these stages are dominant.

Recovery. Four methods for the detection of recovery in this material may be considered. These are (1) comparison of hatchabilities of eggs laid immediately after treatment with those laid later; (2) withholding of females from host for intervals after treatment; (3) fractionation of treatments; (4) changes in time and intensity relationships of treatments. Objections to these methods arise from the facts that (1) not all eggs included in 2a, b and c are in exactly the same stage when treated and

TABLE 5

HATCHABILITY PERCENTAGES FOR EGGS OF TREATED FEMALES MATED TO UNTREATED MALES

Stage treated	Dose in r units	Unmated females		Mated females	
		Number of eggs	Hatchability percentage	Number of eggs	Hatchability percentage
Metaphase I (1a + 1b + 1c)	560	319	39.8 ± 2.7	318	40.5 ± 2.7
Prophase I (2b + 2c)	5,600	137	71.5 ± 2.6	126	70.6 ± 4.0
	22,400	100	19.0 ± 3.9	182	19.2 ± 2.9
Controls	0	127	98.4 ± 1.1	363	98.6 ± 0.6

that this may also be true of 1a, b and c; (2) that almost all eggs have had time for recovery before being laid, if, as Sax (1942) and others believe, recovery takes place within an hour after treatment if at all; (3) that changes within the cell may take place between treatments or during long treatments so that the total dose is not given to any cell at the same stage. However, the data which have been accumulated are herewith presented for what they are worth.

Of twenty-seven experiments in which metaphase I hatchability was studied, with doses ranging from 116 r to 1,500 r, percentages of hatchability were higher in 1b than in 1a for 12, lower for 15; higher in 1c than in 1b for 20, lower for 7; higher in 1c than in 1a for 16, lower for 11. No difference exists between 1a and 1b, but 1c tends to have slightly higher hatchability than 1a or 1b. This presents no valid argument for recovery.

Females were treated and kept for varying lengths of time after treatment before being allowed to oviposit. No delay was great enough to affect hatchability of controls. It is believed that the majority of eggs remain relatively static until laying begins in females under experimental conditions since they have been away from hosts and at 30° C. for twelve hours before treatment and have had ample time in which to store up all metaphase eggs permitted by space or the food supply. Results for eggs treated in metaphase I are summarized in Table 6. Differences are in no case significant and there is no evidence that recovery occurs or that eggs treated in pro-

TABLE 6

TESTS FOR RECOVERY OF EGGS TREATED IN METAPHASE I

Dose in r units	Number of eggs	Interval for recovery in hours	Hatchability percentage
200	167	0	83.1 ± 2.9
200	214	5	87.0 ± 2.3
484	94	0	38.3 ± 5.0
484	93	24	32.3 ± 4.8
500	119	0	38.6 ± 4.4
500	163	4	44.8 ± 3.9
750	196	0	17.8 ± 2.7
750	162	2	17.3 ± 2.9

phase I have entered the egg sacs during the period between treatment and oviposition for hatchability of 1c eggs would be noticeably increased by such a phenomenon. Metaphase I eggs retained for twenty-four hours before being laid have the same length of time for recovery as prophase I eggs in the regular experiments so that time for recovery plays no important part in differential sensitivity.

Data for effects of fractionation on metaphase I are not extensive since but one experiment was carried out to test it. Twelve females were exposed to 700 r and placed on hosts at once. Twelve others were exposed twice to 350 r with a twenty-four hour interval between. Hatchability of 1a, b and c eggs laid by the first was 26.6 ± 4.0, of the second 26.3 ± 5.2. There is no significant difference.

A time-intensity experiment was conducted at the On-

cologic Hospital (Table 7, metaphase I). There are no evidences of any effect of changing time-intensity relationships. Conditions of treatment may have been unsuitable either in size or degree of difference to give evidence of recovery.

The conclusions reached from these experiments are (1) that position in the egg sac appears to have little or no relation to sensitivity and (2) that there is no evidence for recovery of eggs treated in late metaphase I.

Because of the uncertainty as to numbers of chromosome stages among prophase I eggs and as to whether

TABLE 7

COMPARISON OF HATCHABILITY PERCENTAGES FOR EGGS TREATED WITH DIFFERENT INTENSITIES

Stage treated	Dose in r units	Number of eggs	r/m	Time	Hatchability percentage			
Metaphase I (2a + 2b + 2c)	485	182	212	137 s.	53.8 ± 3.6			
	485	119	375	77 s.	56.2 ± 4.6			
	750	230	220	205 s.	30.0 ± 3.0			
	750	245	1,130	40 s.	26.5 ± 2.8			
					2a	2b	2c	2b + 2c
Prophase I	8,661	560	84.9	102 m.	46.4	69.3	80.1	73.8 ± 2.4
	8,661	544	84.9	102 m.	55.9	69.4	61.1	70.3 ± 2.6
	7,803	387	5,600	84 s.	20.8	65.3	67.5	65.8 ± 2.7
	11,200	351	76.5	146 m.	40.6	59.7	62.3	60.4 ± 3.3
	11,200	578	5,600	2 m.	20.3	57.8	64.5	61.0 ± 2.8
	11,200	486	5,600	2 m.	40.5	52.4	68.0	56.7 ± 3.1

these stages remain relatively stable in females after they have stored eggs for twelve hours (although all evidence indicates that they do), the question of recovery in relation to eggs treated in prophase I becomes a more difficult one to attack. Experiments were nevertheless conducted because of the possibility that the effect of fractionation might be greater than could be explained by egg movement from a less to more sensitive stage of vice versa during the intervals between treatments.

Females receiving continuous and fractionated treatments for each dose were bred at the same time. Two-hour intervals were allowed between fractional doses and all groups were kept from oviposition for an hour after the end of treatment so that each irradiated egg would

have at least one hour in which to recover. During intervals between treatments and between final treatment and first oviposition, females were kept at low room temperature in order to retard maturing of eggs and consequent chromosome movement. Females receiving fractionated treatments had four additional hours after the initial treatment in which to permit prophase changes, if such occur. Temperature was kept at 32° C. during oviposition, in contrast to the 30° C. usually employed. Further conditions of treatment and hatchability percentages are presented in Table 8.

TABLE 8

TESTS FOR EFFECTS OF FRACTIONATION OF DOSE ON HATCHABILITY PERCENTAGES FOR EGGS TREATED IN PROPHASE I

Dose in r units	Number of eggs	Time in minutes	Hatchability percentage				
			2a	2b	2c	3	2b + 2c
5,600	360	10	58.3	71.1 ± 4.9	72.2	69.7	71.5 ± 3.8
5,600	606	3–3–4	48.6	74.1 ± 4.0	70.7	67.8	72.9 ± 3.3
22,400	161	4	25.0	15.2 ± 5.2	21.1	8.2	17.8 ± 4.1
22,400	271	1½–1½–1	13.2	36.9 ± 5.9	18.5	12.8	31.5 ± 4.7
22,400	262	4	21.5	18.9 ± 5.3	19.1	4.5	19.0 ± 3.8
22,400	1,131	1½–1½–1	11.8	38.5 ± 3.2	22.4	11.9	35.6 ± 2.8
28,000	452	5	2.3	9.6 ± 2.0	14.0	7.2	10.5 ± 1.9
28,000	581	2–1½–1½	8.1	23.2 ± 4.1	15.3	3.4	19.9 ± 3.0

The difference in hatchability of 2b plus 2c between continuous (71.5 per cent.) and fractionated (72.9 per cent.) for 5,600 r is not significant. For 22,400 r fractionated, hatchability is 13.7 per cent. higher in one experiment (31.5 per cent. – 17.8 per cent.) and 16.6 per cent. higher in the other (35.6 per cent. – 19.0 per cent.). For 28,000 r fractionated, hatchability is 9.4 per cent. higher (19.9 per cent. – 10.5 per cent.). These differences are not great but if the three prophase groups are studied individually, it will be seen that, in fractionated, hatchability is lower for 2a, much higher for 2b and about the same for 2c. The first can be explained by the higher number of eggs laid on day 1 by females receiving continuous treatment. The other two suggest strongly a differential response to fractionation by different stages of prophase I. Data of day 3 show no consistent differ-

ences and are not considered in detail since much oocyte degeneration is going on at this time, especially after the higher doses, and results are not so strictly comparable.

For 22,400 r differences in 2b are 21.7 ± 7.9 and 19.6 ± 6.3; combined, the difference is 21.0 ± 4.8. For 28,000 r the difference is 13.6 ± 4.7. The highest previous hatchability after continuous treatment in 2b for 22,400 r is 15.8 per cent. and the highest in any stage for this dose is 21.0 per cent.; for 28,000 r in 2b it is 9.6 per cent., in any stage 14 per cent.

In view of the fact that there is no evidence for egg movement, as pointed out above, and that, after fractionation, hatchability of 2b is significantly higher than that of the group most resistant to continuous treatment with the same dose, it can be concluded that fractionation has had an effect.

The 5,600 r treatment is not conclusive. There is a difference in hatchability in 2b but it is not significant and earlier experiments (continuous) have given a higher hatchability (76.0) in 2b than this one with fractionation. The difference may be so slight at this lower dose and under the conditions of treatment as to require many experiments to prove it, or there may be no effect. A curve based on hatchability of 2b with fractionated exposures is plotted in Figure 5 where it will be observed that it suggests a straight line; *i.e.*, that a "mixed" curve has been changed by fractionation to a one-hit curve.

Time-intensity variations in treatment are difficult to obtain where such high doses as those necessary for prophase I effects are required. In fact, accurate continuous high doses are impossible to get in most institutions. Experiments here reported (Table 7) give inconclusive results.

From fractionation tests it might be expected that 2b would show the effects of time-intensity differences if recovery is the basis for both. Hatchability of 2b for 7,803 r (high intensity) is lower than for 8,661 r (low intensity) in spite of lower dose. For 11,200 r 2b is like-

wise lower for the higher intensity-shorter time group but in neither case is the difference significant.

Egg-laying "Patterns." It is obvious that, if any environmental factor such as high dose, mating, etc., should alter the egg-laying "pattern," that is, the percentages of total eggs per female which are laid on each day of collection, or the total number itself, the conclusions herein reported would become invalid. Data have been exhaustively analyzed from this angle but a detailed discussion of them would be out of place here. They may be summarized as follows.

. Mean number of total eggs laid on days 1 plus 2 is constant for all experimental conditions used, 18.9–23.5. Mode for each experiment is 24. This constancy appears to be due to the limited and constant food supply, less than could be utilized by the females under any of the experimental conditions. Percentage laid on day 1 plus 2a, $\frac{(1+2a)}{\text{Total}}$, is a constant, as are 2b and 2c. Percentage laid on day 1 varies between experiments but never includes any eggs treated in prophase I. When it is high, 2a is low and vice versa. When percentage laid in 2a is low its hatchability is high, indicating that fewer eggs treated in metaphase I have been retained until day 2. This variability in 2a makes it of no use in this study. Since some eggs almost always hatch in 2a after doses lethal to metaphase I but not to prophase I, 2b plus 2c contains practically none of the former and so this group has been used in estimating prophase I resistance.

Stages at Which Death Occurs with "Lethal" Doses. The persistence with which the Habrobracon egg continues meiosis after irradiation is to be noted. All irradiated eggs which are well enough fixed and stained to show chromatin at all, show a continuation of the meiotic phenomena or an attempt at such continuation after being laid. This is true even after a dose of 200,000 r.

It is difficult to be certain about a comparison of "lethal" doses for eggs treated in each stage. Long and

exhaustive tests would be required to find them exactly. When eggs treated in metaphase I with a dose of 2,016 r and in prophase I with a dose of 44,800 r are incubated for twenty-four hours, fixed and stained, all are seen to have undergone some development and all appear to have died. Of the metaphase I eggs (2,016 r), 2.4 per cent. die in the first cleavage, 7.1 per cent. with a few nuclei, 71.4 per cent. with many nuclei and 19.0 per cent. at blastoderm stage. Of prophase I eggs (44,800 r) 1.1 per cent. die at first cleavage, 30.4 per cent. with a few nuclei, 54.3 per cent. with many nuclei and 14.1 per cent. at blastoderm.

In spite of their relative sensitivity as indicated by lethal dose, eggs treated in metaphase I will continue development after very heavy treatments. All developed to the stage expected (early cleavage) after 4,550 r, some to sixteen nuclear stage (expected) after 15,000 r, to metaphase II after 25,000 r, to pronucleus after 35,000 r and one to anaphase II after 200,000 r. At doses of 4,550 r and lower there is no apparent slowing up of development. At 15,000 r some appear to develop at normal rate (with ten minutes variation). No records of time were kept at the higher doses and no observations were made on prophase I with doses higher than 44,800 r.

CYTOLOGY

A summary of the cytological effects is given in this paper in order that use may be made of all available information in the discussion. A detailed account with drawings is in preparation. The extremely small size of the chromosomes and their relatively large number should be kept in mind by the reader who tends to be critical of the limited observations. About 2,500 eggs were prepared and studied.

Technique. Eggs from control and treated females were incubated according to control schedules, dropped into fixative (formalin-acetic-alcohol), punctured at the posterior end to facilitate fixation, treated with the

Feulgen nucleal-reaction and mounted whole in balsam. Whole mounts were made of ovaries with comparable technique. Control hatchability tests were made of eggs treated at the same time as those fixed.

Meiosis in Controls. The majority of students of hymenopteran cytology would question the statement that the stored oocyte is in metaphase I. They state that the chromatin has reverted to a resting stage, or has formed an abortive maturation spindle or a compact clump or a composite body, etc. Speicher (1936) states that the most advanced eggs in the Habrobracon egg sac are in "early anaphase of the first maturation" and "further progress in maturation is stopped until after oviposition." The author prefers to call this late metaphase of the first maturation and has checked repeatedly the observation of Speicher that distinct chromosomes are present, are in the form of tetrads and are ten in number. The conclusion must be drawn that Habrobracon differs from many other Hymenoptera in having meiosis uninterrupted between metaphase I and anaphase I by an "amorphous" or "compact" clump of chromatin, or that its chromosomes, in spite of their small size, must retain more easily their individuality when fixed.

The meiotic stages as described by Speicher will be outlined briefly. For several minutes after being laid the maturation spindle appears much as in the unlaid egg except that it has been moved from the dorsal to ventral side of the egg during oviposition. It then rotates to a position vertical to the egg surface and passes into telophase I. The second division follows immediately. The four groups of chromosomes (1a, 1b, 2a, 2b) lie in a row roughly perpendicular to the egg surface. During anaphase II polar nuclei 1a and 2a remain stationary, 1b moves close to 2a, and 2b (ootid nucleus) sinks deeper into the egg, a nuclear membrane forming as it moves. Nucleus 1a quickly disintegrates, 1b and 2a unite, form a metaphase plate, which divides and disintegrates.

Meiosis in Eggs Irradiated in Metaphase I. Chromosomes in eggs just laid show no effects of irradiation.

The dyads are distinct and appear to be uninjured. Effects of treatment are first visible in anaphase I when fragments can be seen within the spindle and between the chromosome groups. These are often almost as large as the chromosomes and usually appear to be double. They are considered terminal deletions. No evidence of a bridge has been seen in any of the large number of eggs studied. In subsequent meiotic stages these fragments can still be seen and counted. In division II, fragments, small and apparently single, may be observed as well as bridges or both.

The number of fragments in division I varies with dose as does the number of eggs with fragments (Table 9).

TABLE 9

COMPARISON OF HATCHABILITY PERCENTAGES AND OF CYTOLOGICAL OBSERVATIONS FOR EGGS TREATED IN METAPHASE I

Dose in r units	Hatchability percentage	Percentage of eggs without deletions	Hatchability percentage estimated	Deletions per egg	Power
500	50.0	37.0	62.9	1.0	...
1,000	21.0	15.3	38.4	1.92	0.94
1,500	7.1	6.1	24.2	2.39	0.53
2,000	0.0	4.2	17.0	2.84	0.58

In Figure 6 are plotted the dose-hatchability curve for eggs irradiated at the same time as those fixed and the curve for the percentages of eggs without deletions. It is apparent that some eggs with deletions must hatch. If there is but one deletion in an egg and it is double and terminal, the ootid nucleus would have a 1:1 chance of receiving an unbroken chromatid since the injured dyad may go to either pole in division I and there is no bridge in this division to prevent a broken chromatid from entering the ootid nucleus (Beadle and Sturtevant, 1935). If there are two broken dyads the ootid nucleus would have one chance in four of being free from injury. This will explain discrepancies between these two curves in Fig. 6 and furnish a reason for their approaching each other at high doses when most eggs have several deletions.

The effects of 500 r and of 1,000 r vary as the 0.94 power of dose. As dose increases this power becomes

smaller (Table 9). This can be explained by supposing either that deletions become smaller as they become more numerous and can not be seen so readily or that when a deletion is once free from the attached portion of the chromosome further injuries in it undergo restitution. The latter has been tested recently by Sax (1942) who finds that effective breaks are much more frequent in the centric chromosomes than in acentric fragments.

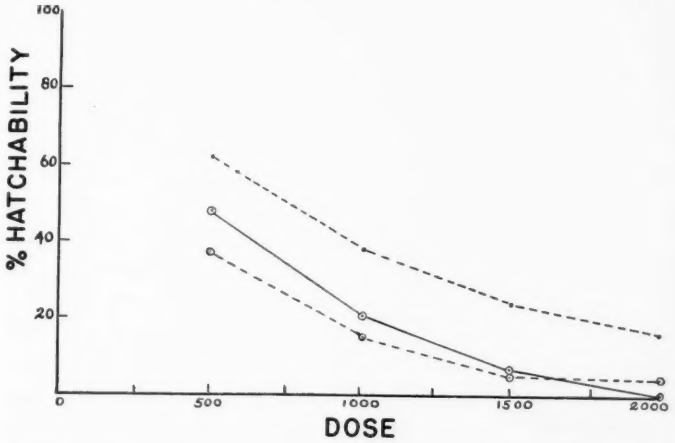

FIG. 6. The relation between x-ray dose in r units and (1) hatchability percentages (solid line), (2) percentages of eggs without fragments in telophase I (lower dotted line) and (3) percentages of eggs containing no fragments plus $\frac{1}{2}$ containing one and $\frac{1}{4}$ containing two, a "theoretical" hatchability curve (upper dotted line). Eggs were treated in late metaphase I.

Bridges in division II are found more frequently between nuclei 2a and 2b (ootid nucleus) than between 1a and 1b. Since nucleus 1a disintegrates very soon after it is formed, the attached monad might return to 1b when freed in the disintegrating nucleus and bridges in the outer spindle would be temporary. The ootid nucleus forms a wall as it moves toward the center of the egg. When a bridge is present this nucleus becomes shaped like a tear drop and then spherical with a small knob at

one side, connected by the bridge to 2a. The ductility of the bridge and its resistance to breakage is remarkable.

Meiosis in Eggs Irradiated in Prophase I. Eggs irradiated in prophase I show the same evidence of injury as those just described except that bridges may be formed in division I. These are not frequent enough, however, to explain high resistance of this stage (Beadle and Sturtevant, 1935). No detailed statistical analysis of injuries to chromosomes treated in prophase I has been made as yet. At low doses (500 r to 2,000 r) 3.4 per cent. of the eggs have shown injury. Only one of these was a bridge in division I. At 15,000 r (30 per cent. hatchability) 36.4 per cent. show injury. In this group as well as that treated with 42,000 r (almost lethal) the same types of injury occur. When a bridge is present in division I it is often, if not always, permanent and when nucleus 1b moves toward 2a in division II the bridge can be seen as a loop bulging at the side and connecting nuclei 1b and 2a.

Cleavage in Irradiated Eggs. Bridges have been found in cleavage I in eggs treated in both stages indicating that, if chromatids were already split when treated, there had occurred lateral fusion of broken ends. If they were not split when treated the split must have been "incomplete" when it occurred in the broken chromatid after treatment. Bridges and fragments appear frequently in cleavage and the latter are formed by two breaks in a bridge which release a thickened mid-region of the bridge. Such fragments are pointed at each end in contrast to those observed in meiotic divisions.

DISCUSSION

Muller (1940) states that "eggs and young embryos tolerate a much lower dose than sperm, yet they do not have as many genetic changes induced in them." If, by toleration of lower dose, is meant lower lethal dose and, by genetic changes, a term broad enough to include dominant lethal effects, neither part of the statement holds for

Habrobracon. Dominant lethal effects are induced in all eggs treated in metaphase I at about 1,400 r, in spermatozoa at 10,000 r and in eggs treated in prophase I at 45,000 r. Spermatozoa are not actually inactivated by doses much larger than 10,000 r but neither are eggs by doses much larger than lethal since they continue meiotic phenomena under such conditions. These facts strongly support Reynolds (1941) in his conclusion that the sex of the cell has nothing to do with its response to x-rays but rather that the condition of the chromatin determines cell sensitivity.

The extreme and unexpected sensitivity of metaphase I in Habrobracon oocytes has caused some investigators to suggest that the effect is "physiological" and not due to direct chromosome change. The attention of the author was called to the work of Lamy and Muller (1939) who show that diploid Drosophila embryos are no more sensitive than triploid at the same x-ray doses, an argument against chromosome change and, therefore, in favor of "physiological" effects. The author (Whiting and Bostian, 1931) found that haploid (male) larvae are more sensitive to irradiation than diploid (female) larvae of Habrobracon, as expected if chromosome change is responsible. These are normal forms in this species in contrast to the anomalous triploids in Drosophila.

Sax and Swanson (1941) describe the clumping and sticking together of metaphase chromosomes in Tradescantia microspores after irradiation due to "physiological" effect. Such an effect, if produced in metaphase I oocytes of Habrobracon, must be very transitory. Eggs treated in this stage and in prophase I all have chromosomes and fragments, when present, distinct in outline in stages following oviposition and no fusion bridges have been seen. Absence of a low dose threshold for injury and absence of a high one for clumping and retardation of meiosis and early cleavage for doses up to 15,000 r furnish additional evidence against any serious "physiological" effect in this stage. Since both metaphase I

and prophase I eggs, after their respective lethal doses, present the same pattern in regard to degree of development before death, causes of death would appear to be of the same nature for each.

The fact that the lethals induced in these oocytes are dominant has been used as an argument for "physiological" injury. This is inconsistent with the view held by all investigators in this field, that dominant lethal effects in spermatozoa of Drosophila are chromosomal.

The linear proportionality between dose and hatchability as well as the other facts about metaphase I oocytes outlined above can be explained by the following hypothesis. At the time of treatment, dyads are under tension since spindle fiber regions have started toward the poles while ends are still held in contact by chiasmata. When a hit breaks a pair of chromatids (a dyad) proximal to a chiasma the parts will separate, too far to rejoin but not far enough for the fracture to be seen. Subsequent complete terminalization and separation of dyads in anaphase I leave fragments and there are no bridges. A broken dyad produces a bridge in division II in either spindle with equal frequency. If a single chromatid is broken the fragment remains attached to its uninjured partner and appears in division II (McClintock, 1938). An egg with a single deletion will fail to hatch if unfertilized, and, even if fertilized, when the deletion is large. If two chromatids in a "lug" (distal to chiasma) were broken and the chromatids fused laterally, an occasional bridge in division I might be expected. Tension is much less in this region so that restitution usually occurs when a hit causes a break in this short length of chromosome.

As explained above, an egg with one double fragment has one chance in two of hatching, an egg with two such fragments, one chance in four (if the possibility of two breaks in one dyad be left out of consideration). A "hatchability" curve can be made by adding to the eggs showing no fragments, one half of those showing one, and one fourth of those showing two. Such a curve (Figure 9)

is considerably higher than the actual hatchability curve of treated controls but parallel to it. Probably if all deletions could be seen and the theoretical hatchability curve constructed, it would coincide with the actual curve, or lie very close to it.

These data conform with the conclusions of Sax (1938), Belgovsky (1939), Marshak (1939), Faberge (1940), Carlson (1941), and Pontecorvo (1941) in respect to one-hit phenomena but appear to differ from those of Newcombe (1942).

Sax (1940) found that a single hit usually does not break sister chromatids in or after late prophase and Muller (1940) is not convinced that double breakage from a single ionization has been proved in any form. Delbrück (1940) questions the production of two breaks by a single ionization on physical grounds. In Habrobracon, bridges in division II following treatment in late metaphase I can not be explained by chromosome splitting following breakage in view of the structure of the metaphase tetrad. It therefore seems probable that a single hit has broken two chromatids in late metaphase I.

The prophase I hatchability curve (Figs. 3, 4, 5) does not show a linear relation to dose. Rather, it resembles the dominant lethal curve for Drosophila sperm as described by Fano and Demerec (1940). "The production of dominant lethals is apparently proportional to the x-ray dose for small dose and the survival ratio decreases exponentially for high dose. On the whole, however, the 'dose-action' curve does not seem to fit the 'single hit' type very well. This would indicate that the phenomenon is not simple, which is very plausible." More than one stage is included in prophase I and undoubtedly aberrations of different kinds are induced.

When hatchability of prophase I exposed to fractionated treatments is plotted semilogarithmically (Fig. 5) an approximate straight line is formed and the modified curve stands in contrast to that of high continuous doses where a speeding up effect appears. Sax (1942) has found that

fractionated dose decreases the frequency of complex aberrations. He attributes this to restitution between exposures. The elimination of a large proportion of complex rearrangements might change a "mixed" curve into a one-hit curve. The variation in hatchabilities at dose 16,800 r, the point near which the trend of the curve seems to change, may indicate the neighborhood of a threshold at which a new phenomenon enters the picture. Doses above and below this point were chosen for fractionation tests with the idea that lower dose might not respond to fractionation (one-hit part of curve), that high dose might do so (mixed part). Results do not contradict this. If Newcombe's (1942) suggested delayed restitution at high doses begins to take effect in the neighborhood of 16,800 r for prophase, and repeated smaller doses have no cumulative effect of this type, an increase in hatchability with fractionated treatments would be expected only at higher doses.

The period of high sensitivity of prophase I, late prophase (latest prophase I can not be estimated because of mixture with metaphase I eggs) is the same as that which responds to fractionation. The higher sensitivity might be explained by supposing that terminalization, beginning in this stage, would increase breakage because of increased tensions. An explanation for a relationship between terminalization and fractionation effects seems difficult to formulate. A detailed discussion of the cytological basis for prophase I response to x-rays would be of little value without further study of the chromosomes. Obviously, broken ends of sister chromatids fuse, if bridges in division II are an indication of this. It is suggested that, although ionizations may injure these prophase I chromosomes as frequently as they do the later more condensed ones, the injuries are temporary and restitution is high because of the small amount of movement, and therefore of tension, at time of treatment. They ultimately undergo all the movements and tensions of those treated in metaphase I and also additional ones

connected with their condensation and arrangement on the metaphase plate but they emerge unmodified, or with non-lethal changes, unless treated with very heavy doses.

It is known that many deficiencies are viable in the heterozygous condition, lethality being dependent on gene content of the segment lost. Since diploid larvae are more resistant to irradiation than haploid, recessive deficiencies must be induced in somatic cells. It is surprising, in view of these facts, that all the lethal effects of irradiation herein described appear to be dominant. If breaks proximal to the chiasmata are the ones which tend to be permanent, these would necessarily result in large (and therefore dominant) terminal deletions. Deletions small enough to act as recessives might not have a lethal effect on the haploid embryo until after hatching and therefore would not be detected with methods used in the present work. In the limited tests made with fertilized eggs it is also possible that a few deficiency heterozygotes surviving would not noticeably affect hatchability ratios.

Attempted explanations of differential sensitivity to x-rays may be classified into those that involve differences (1) in original breaking of a chromosome strand (*true* difference in sensitivity), (2) in number of strands broken per ionization and (3) in restitution.

Marshak (1937) advanced the first of these and Darlington (1942) and Sax and Swanson (1941) have since suggested that high nucleic acid content, as in metaphase chromosomes, would prevent breakage. This argument can not apply to results herein reported.

Sax (1940) suggests the second, that, as number of strands in a chromosome increases, breaks per ionization will increase and (Sax and Swanson, 1941), as chromatids become more widely separated, one only will be broken by an ionization. In the tetrad breakage of a chromatid with two strands would not differ in effect from breakage of one with a single strand since the chromatid acts as a unit until time of first cleavage. In both prophase I and

metaphase I lateral sister chromatid fusion takes place, and, presumably, breakage of two chromatids by one hit.

Factors which might inhibit restitution in one stage and not in another are (a) lateral sister chromatid fusion (Sax and Swanson, 1941), (b) movement of chromosomes (Metz and Bozeman, 1940; Sax, 1940; Wilson and Nebel, 1939; Muller, 1940), (c) tension (as distinct from movement), (Goodspeed, 1929; Stone, 1933; Mather, 1934; Sax and Swanson, 1941), (d) time difference between treatment and division (Stone, 1933; Lea, 1938), (e) retardation factor late in cycle (Newcombe, 1942).

In respect to Habrobracon eggs (a) there is lateral sister strand fusion in both stages; (b) chromosomes in more resistant stage probably move more than in sensitive; (c) tension appears greater in sensitive stage; (d) all eggs treated in prophase I and most of those treated in metaphase I have more than one hour for recovery and when each stage is given identical long periods for recovery there is no evidence of effect; (e) if twenty-four hours are allowed for restitution in some metaphase I eggs while others with same treatment are laid at once, restitution still does not take place in former, lateral sister chromatid fusion has taken place in both. If Newcombe's factor slowing up restitution late in cycle exists, it does not prevent relatively rapid lateral sister chromatid fusion and so can not be due to any inabilty of injured ends of chromatids to undergo fusion.

It must be concluded that tension and its influence upon restitution is the most convincing explanation for the results herein reported. With this must be included a relatively rapid recovery in chromosomes treated in prophase I. That the degree of tension is due to conditions peculiar to the first meiotic division seems probable.

SUMMARY

A. Unlaid Habrobracon eggs x-rayed in late metaphase I and allowed to develop parthenogenetically

(1) Show a linear decline in hatchability from 50 r to 1,400 r, approximately the lethal dose.

(2) Show no change in hatchability percentages with aging after treatment, time-intensity differences or fractionation of dose.

(3) Continue meiosis and early cleavage after doses several times lethal, at rates not noticeably different from controls.

(4) Show fragments but no bridges in division I, bridges, fragments or both in division II, fragments and bridges in cleavage.

(5) Give a curve expressing dose-percentages of eggs without fragments which at high doses converges toward the dose-hatchability curve.

(6) Show a number of fragments per treated egg which varies as the 0.94 power of dose between 500 r and 1,000 r, 0.53 power between 1,000 r and 1,500 r, and 0.58 power between 1,500 r and 2,000 r.

B. Unlaid eggs x-rayed in prophase I and allowed to develop parthenogenetically

(1) Give hatchability percentages equal to or higher than controls from 50 r to 750 r.

(2) Show a decline in hatchability from 1,000 r to 44,800 r, approximately the lethal dose.

(3) Give a hatchability curve which appears to be linear at low doses and to become "mixed" at high doses.

(4) Show no significant change in hatchability in preliminary test of time-intensity differences but an increase with fractionation of dose.

(5) Continue meiosis and early cleavage at lethal dose at rates not noticeably different from controls.

(6) Show fragments and bridges in division I, either or both in division II, fragments and bridges in cleavage.

C. Unlaid eggs treated in either stage

(1) Show no change in hatchability when fertilized by untreated spermatozoa.

(2) Develop as far as blastoderm in about twenty per cent. at approximately lethal doses.

D. It can be concluded that

(1) Consistent and correlated cytological and hatchability patterns of eggs treated in late metaphase I appear

to prove conclusively that death is due to chromosome breaks arising from single hits.

(2) Consistent differences in cytological effects and in nature of dose-hatchability curves correlated with consistent differences in stages of chromosomes at time of treatment argue strongly for the dependence of ionization effects upon the physical (or chemical) condition of the chromosomes.

(3) High sensitivity of metaphase I, the stage in which tensions of chromosomes have been considered very high, argues strongly for the dependence of ionization effects upon the tension of chromosomes. Other suggestions made to explain differential sensitivity of chromosomes in different stages do not apply to material used in this study.

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CHROMOSOME STUDIES ON EIGHT SPECIES OF SCIARA (DIPTERA) WITH SPECIAL REFERENCE TO CHROMOSOME CHANGES OF EVOLUTIONARY SIGNIFICANCE. II

(*Continued from page 121*)

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Sciara species VII

1. SOURCE AND GENERAL CHARACTERISTICS

This species was collected in each of two greenhouses within five miles of each other. The laboratory stock was derived from the offspring of two females collected at the same time in a greenhouse located in Newtown Square, Pa. Individual mated females of this species produce offspring of predominantly one sex.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic chromosome group (Fig. 1b) is composed of three pairs of rod-shaped chromosomes and one pair of V-shaped chromosomes. The gonial metaphase group (Fig. 1a), on comparison, shows four pairs of rods, one pair of V's. Of these chromosomes, two rods are without homologues in the somatic complex, and are considered to be the "limited" chromosomes.

3. SALIVARY GLAND CHROMOSOMES

The salivary gland chromosomes of this species are very difficult to spread and stain. Various modifications of the technique used for the other species were tried without success. Slides of more than eight hundred larvae were made in order to obtain four good preparations and a dozen fair ones.

A great deal of asynapsis and subsequent twisting of the two homologues about each other made the accurate determination of band pattern very difficult. The question whether asynapsis of the two homologues is always accompanied by a band pattern difference can be answered in the negative according to the evidence presented by this species. A short region of the C chromosome (Fig. 25b) is not synapsed and shows no pattern

difference. Likewise, in the two short regions shown in Fig. 28b the banding patterns are the same. These cases do not rule out the possibility of submicroscopic differences which may be responsible for the asynapsis.

Small differences between homologues. The arrow in Fig. 25a (end 1 of the C chromosome) points to a vesicular band in one homologue which is apposed to a non-vesicular band in the other homologue. This kind of small difference was also found in another species to be mentioned later. End 2 of the C chromosome shows no irregularities (Fig. 25c). End 2 of the A chromosome (Fig. 28b) shows a split end and a terminal deficiency marked by an arrow, in addition to three short asynaptic regions in which no pattern difference can be detected.

"*Repeats.*" There were no "repeats" observed in this species. The X chromosome, which has shown "repeats" in other species, is regular (Fig. 26).

Rearrangements. A short intercalary inversion of at least three bands was found in end 1 of the B chromosome (Fig. 27a). A similar inversion was found in end 1 of the A chromosome (Fig. 28a). These two inversion figures have been diagrammed in Fig. 27b. The asynapsis in end 1 of the A chromosome (Fig. 28a) does not seem to be due entirely to the inversion, but partly to a lack of band homology. As has been shown above, the C chromosome of the laboratory stock did not carry any inversions. But on examination of the slides made from the second collection of this species, the C chromosome was found to be heterozygous for the subterminal inversion shown in Fig. 25e. This collection was made in a greenhouse at Media, Pa. The entire figure of this inversion is diagrammed in Fig. 25d.

Sciara nacta Johannsen

1. SOURCE AND GENERAL CHARACTERISTICS

This very small species seems to be widely distributed in greenhouses, having been found on numerous occasions as far north as Springfield, Mass., and as far south as Tampa, Fla. The laboratory stock was derived from the offspring of a female collected at Gradyville, Pa.

Mated individual *S. nacta* females produce bisexual progenies. The species thrives well in the laboratory and may be kept in mass cultures without difficulty.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase chromosome group is composed of four pairs of rod-shaped chromosomes. Fig. 26 shows a somatic group with the homologues of three pairs separated. The gonial metaphase group (Fig. 2a) shows four pairs (homologues separated) of chromosomes homologous with those of the soma. In addition, there are two large unpaired, rod-shaped chromosomes. These are the "limited" chromosomes. This is the third species in which there has been found two unpaired rod-shaped "limited" chromosomes. The possible significance of this fact will be treated in the discussion.

3. SALIVARY GLAND CHROMOSOMES

Although the individuals of this species are small, the salivary gland chromosomes are very favorable for study. They smear and stain readily, giving, for the most part, sharply banded figures. The chromosome ends are easily differentiated.

Fig. 29a shows end 1 of the C chromosome. Fig. 29b shows end 2 of the C chromosome with its characteristic "puff." Figs. 31a and 31b are the two ends of the A chromosome.

Small differences between homologues. Only one consistent small difference was seen in this species. Fig. 30a (end 1 of the B chromosome) shows a small difference, indicated by the arrow, in which a single band in one homologue is apposed to a thick band in the other homologue. End 2 of the B chromosome is regular (Fig. 30b).

"Repeats." End 1 of the X chromosome (Fig. 32a) is characterized by a bifurcated tip and a subterminal knotting of the chromosome approximating a figure eight. This latter condition is due to a double "repeat" in which the synapsis of three linearly separated homologous regions has occurred. No cells were found in which the band pattern of these three regions was clear. The

banding pattern of end 2 of the X chromosome is seen in Fig. 32b.

Rearrangements. No rearrangements were found in the laboratory stock from which the above data were compiled. However, two short inversions were found, one in each of two other stocks. Fig. 29c shows one of these, which is a short subterminal inversion in end 1 of the C chromosome. The other is a short subterminal intercalary inversion of end 1 of the C chromosome (Fig. 29d). This figure also shows (see arrow) a terminal deletion in which one homologue is minus its terminal band.

Sciara fenestralis Zett., form II

1. SOURCE AND GENERAL CHARACTERISTICS

This species was found in a number of greenhouses within a fifty-mile radius of Philadelphia and also in a meadow in Ridley Park, Pa. Individual mated females of this species produce unisexual progenies in which occasional individuals of the opposite sex are found. The laboratory stock was derived from the mating of male and female offspring of two females captured in a greenhouse at Newtown Square, Pa.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase group (Fig. 7b) consists of two pairs of V-shaped chromosomes and two pairs of rod-shaped chromosomes. In addition to those chromosomes which are homologous to the somatic metaphase chromosomes, the gonial metaphase group has one large V-shaped "limited" chromosome (Fig. 7a).

3. SALIVARY GLAND CHROMOSOMES

The salivary gland chromosomes of this species offer no technical difficulties, but they twist about each other and have a high degree of asynapsis, making it difficult to be sure of band pattern. The X chromosome is the only one of the complex which shows no irregularties. Ends 1 and 2 of the X are seen in Figs. 35a and 35b, respectively.

Small differences between homologues. The A chromosome is exceptionally long and twisted. End 1 is shown in Fig. 33a. End 2 (Fig. 33c) has a short subterminal asynaptic region (see arrow) in which an optically homogeneous region in one homologue is apposed to a banded region in the other. Fig. 33b, a short central region of the A, shows a single band apposed to a vesicular band. Two other small differences were found in the inverted C chromosome. One of these, marked by the arrow in Figure 36a, shows a thin band in one homologue apposed to a thick band in the other. The second is seen opposite the arrow in Fig. 36b. In this case, a pair of thin bands is apposed to a large vesicular band.

"Repeats." No "repeats" were found in this species.

Rearrangements. Fig. 33d shows a short intercalary inversion of the A chromosome. This is the same kind as that diagrammed in Fig. 27b. The B chromosome (Fig. 34) is heterozygous for a subterminal inversion of about two thirds of its length. Within this long inversion, there is a short inversion as shown in the figure. The C chromosome (Fig. 36b) is heterozygous for a nonterminal inversion involving about three fourths of the chromosome. Fig. 36a shows the banding pattern of the synapsed ends of the C chromosome which are not shown in Fig. 36b.

Sciara fenestralis Zett., form I

1. SOURCE AND GENERAL CHARACTERISTICS

This material was collected in a greenhouse at Colmar, Pa. The laboratory stock was derived from the offspring of a single female, since the species produces bisexual progenies. The sex ratio in this species always favored the males. The longer the stock was kept in the laboratory the greater the preponderance of males. In the eighth month after collection, the females cast only males and the stock was lost. This species is closely related to *S. fenestralis,* form II, as stated above. Although mating reactions were observed between the two species, no offspring were ever produced from reciprocal matings.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase chromosomes (Fig. 4b) consist of two pairs of V-shaped chromosomes and two pairs of rod-shaped chromosomes. The gonial metaphase group (Fig. 4a) has two large V-shaped chromosomes which have no homologues in the soma. These are the "limited" chromosomes.

3. SALIVARY GLAND CHROMOSOMES

Although the somatic metaphase chromosome groups of *S. fenestralis*, forms I and II, are very similar, the banding pattern of the chromosome ends of the two species is different (figures on plate V). In the material studied, the salivary gland chromosomes of form I show fewer aberrations than those of form II, although form I has a "repeat" in the X chromosome which form II lacks.

Figs. 40a and 40b show ends 1 and 2 of the B chromosome; these show no asymmetry.

Small differences between homologues. End 1 of the A chromosome (Fig. 39a) shows no aberrations, but end 2 of the B chromosome (Fig. 39b) shows a small difference marked by the arrow. In this case, a thin band is apposed to a thick one. Another small difference is seen opposite the arrow in the synapsed ends of the C chromosome (Fig. 38b). In this latter case, there is a thin band in one homologue which has no counterpart in the other.

"Repeats." The X chromosome possesses a "repeat" of at least three bands causing it to loop back on itself when the two homologous regions synapse (Fig. 37b). However, it is difficult to be sure that only three bands are repeated. Fig. 37a shows the pattern of end 1 of the X which does not show clearly in figure 37b. Fig. 37c shows end 2 of the X chromosome.

Rearrangements. Fig. 38a shows a subterminal inversion involving about five sixths of the chromosome. Fig. 38b shows the band pattern of the short free ends which do not show in Fig. 38a.

DISCUSSION

1. GONIAL AND SOMATIC GROUPS

Since the genus *Drosophila* and the genus *Sciara* are included in the order Diptera, but widely separated, and since they can be studied by comparable methods, it is important to investigate any significant differences between them in chromosome organization, in the hope that some light may be shed on the evolutionary processes at work. For the most part, the writer can only point out in this paper certain similarities and differences. The full significance of the data will not be clear until further studies can be made on *Sciara.*

It is of interest to note that all the species of *Sciara* thus far examined have four pairs of chromosomes in the soma. In addition to the chromosome groups presented in this paper, those of *S. coprophila, S. similans, S. pauciseta* and *S. prolifica* were published by Metz (1926a, 1926b), and those of *S. reynoldsi* and *S. ocellaris* were published by Crouse (1939). Mr. Carson is preparing for publication figures of *S. impatiens.* Hence, the chromosome complexes of a total of fourteen species have been examined, all of which show four somatic pairs of chromosomes.

The forms of these four pairs, however, differ and fall into three groups as pointed out above. Of the fourteen species, two have all rod-shaped chromosomes, four have three rod-shaped pairs and one V-shaped pair, and eight have two rod-shaped and two V-shaped pairs. There is at the present time no indication which, if any, of the above groups is the ancestral type. One species in each of the three groups is widely distributed; namely, *S. ocellaris,* two V's—two rods; *S. impatiens,* one V—three rods; *S. nacta,* four rods. The method of change from V to rod by shift of the spindle fiber locus and *vice versa* has been postulated and discussed by Crouse (1939).

Evolutionary changes in the "limited" chromosomes have been discussed by Metz (1938b). It seems desirable to summarize at this time the occurrence and nature of these bodies in the fourteen species studied to date.

Three of the fourteen species have no "limited" chromosomes. Metz postulates that these probably represent the more recent species. Of the remaining eleven species, three have been found to have two rod-shaped "limited" chromosomes each, and eight have been found to have V-shaped "limited" chromosomes. Metz (*op. cit.*) has discussed the fact that those species possessing "limited" chromosomes may have more than one V-shaped "limited" chromosome in some individuals but never less than one. Hence, the assumption is that the "limited" chromosomes in those species possessing them do have some necessary function.

The numbers of the species representing the three different conditions with respect to "limited" chromosomes suggest that the V-shaped "limited" chromosome is the ancestral type. The presence of three species without "limited" chromosomes which are otherwise the same in chromosome number and general behavior leads to the supposition that the "limited" chromosomes are gradually being lost from this genus. Additional evidence on the form and number of "limited" chromosomes is presented here from three species, each of which has two rod-shaped "limited" chromosomes. In each of these cases, the rod-shaped "limited" chromosomes appear to be morphologically identical. Such a condition could conceivably arise by a fragmentation through the apex of an equal armed V-shaped chromosome.

Since those species found to have rod-shaped "limited" chromosomes always have two of them, the question arises concerning the genetic activity of each. If these rods do represent halved V-shaped "limited" chromosomes, then it seems probable that the genetic activity of the V-shaped chromosome is distributed on either side of its apex or centromere. If this were not so, one would expect to find some races or species possessing a single, short rod-shaped "limited" chromosome. Observations on members of three different races of *Sciara species* XXIII showed no single rod-shaped "limited" chromosomes. Further investigation is needed to determine (1) whether species exist in nature with only one rod-

shaped "limited" chromosome, (2) whether those species possessing two rod-shaped "limited" chromosomes could survive with only one.

2. SALIVARY GLAND CHROMOSOME CONSIDERATIONS

Rearrangements. Five of the eight species reported in this paper show chromosome rearrangements similar to those found in *Drosophila* (for a review of this subject see Muller, 1939) and *Chironomus* (Bauer, 1936). In addition to these, three other species, making a total of eight out of fourteen species, show gross chromosome rearrangements. Hence, this genus can not be said to differ fundamentally in this respect from *Drosophila* or *Chironomus.*

The species for which no rearrangements have been recorded are certainly not to be considered different in this respect on the basis of the evidence on hand. This point is illustrated by the fact that each of two different collections of *S. ocellaris* in Delaware County, Pa., yielded a large inversion, whereas previous collections had not done so.

The preliminary observations on the *S. ocellaris-reynoldsi* species hybrids, like the *Chironomus* hybrids reported by Goldschmidt, 1942, showed no typical rearrangement figures (Metz and Lawrence, 1938). This led to the thought that possibly such changes did not occur in *Sciara.* However, the work of Dr. Pauline Rohm (unpublished) analyzing and mapping the *S. ocellaris-reynoldsi* hybrid C chromosome has shown that the two homologues differ by two inversions.

"Repeats." The subject of "repeats," their method of origin and their probable significance has been discussed by Bridges (1935). The "repeats" on which Bridges based his discussion were found in *Drosophila melanogaster* autosomes. Metz and Lawrence (1938) published photomicrographs of "repeats" as seen in *Sciara ocellaris* and in *S. ocellaris-reynoldsi* hybrids. These "repeats" were in the X chromosomes. The "repeats" found in six of the eight species reported in this paper have all been present in the X chromosomes. The

significance of this phenomenon has not yet been discovered.

According to the postulates of Bridges, these "repeats" probably arise through duplication and then subsequent translocation of the duplicated region within the chromosome. The "repeats" studied in this paper would tend to bear out this theory, for the sizes of the loops in "single repeats" vary from a tight loop in which the repeated regions are adjacent, Fig. 10a, to loose loops of different sizes, Figs. 13a, 24b and 37b. A similar situation obtains in the "double repeats," as shown by Figs. 19b and 32a. The banding pattern of the repeated regions is difficult to obtain clearly, and thus far the writer has not been able to demonstrate any similarity of pattern among the repeated regions of the several species studied.

Small differences. The nature and frequency of occurrence of small differences in the banding pattern of five of the eight species studied here are different from those recorded for *Drosophila.* Hence, there is reason to believe that these represent a phenomenon which is characteristic of the genus *Sciara.* The differences between homologues pointed out above in the observations can be classified into five categories; namely: (1) "single" band deficiencies, (2) vesicular band apposed to disc-shaped band, (3) thick band apposed to thin, (4) homogeneous region apposed to banded, (5) terminal band deficiency.

The first, third and fifth of these categories have been discussed for *Sciara ocellaris* and the findings compared with those of other investigators by Metz (1937, 1938a). The second category is one not found in *Drosophila,* but was represented in this material by two cases. The third category was most numerous, being represented by four cases. Possibly these cases are ones in which the thick bands represent a duplication of material, thus providing for chromosome enlargement. The fourth category was found in several cells from different individuals of one species. That a real difference exists here between the homologues is indicated by the asynapsis. The fifth

category was found consistently in individuals of only one race of one species. This type of deficiency was described by Kikkawa (1938) for *Drosophila ananassae*. Kikkawa was of the opinion that in his material these apparent terminal deletions were not deletions but represented additions on one homologue. He found no subterminal cases of this or any other type of small difference in his material and concluded that none existed.

Taxonomy. The taxonomy of the genus *Sciara* is at the present time in a confused state. There exists considerable overlapping between American and European species, and, indeed, there exists some discrepancy between the existing key to the species and the description of some of the species therein (Johannsen, 1917). In order that at some later time names may be given to those species which I have referred to by number and that those which I have called by name may be checked, I am including a photomicrograph of the right wing of the male of each species (Plate VI) and an outline drawing, drawn with the aid of a camera lucida, of the gross morphology of the hypopygium of the male of each species as seen from the ventral surface (Plate VII).

Pinned specimens and alcoholic specimens of each of the species have been deposited with Dr. Alan Stone at the U. S. National Museum, Washington, D. C.

SUMMARY

1. Eight different species of *Sciara* have been captured in the wild and reared in the laboratory.

2. Somatic chromosome complexes of all eight species show four pairs of chromosomes, which fall according to shape into three categories: (a) All rods, two species; (b) Three pairs of rods—one pair of V's, two species; (c) Two pairs of rods—two pairs of V's, four species.

3. Gonial metaphase figures show four species to possess V-shaped "limited" chromosomes, three species to possess rod-shaped "limited" chromosomes and one species to possess no "limited" chromosomes.

4. Salivary gland chromosome studies disclose the following: (a) Six of the eight species have "repeats" in

the X chromosome, of which four are single and two are double; (b) Five of the eight species show chromosome inversions of varying complexities and lengths; (c) Six of the eight species show "small differences" between homologues in the salivary gland chromosome banding which are seemingly characteristic of the genus.

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EXPLANATION OF FIGURES, PLATE IV

25–28 *Sciara species VII.* Salivary gland chromosomes.

25a End 1 of "C" chromosome. Arrow indicates a vesicular band whose homologue is nonvesicular.

25b Intercalary region of "C" chromosome in which there is asynapsis with no visible band difference.

25c End 2 of "C" chromosome.

25d Diagrammatic representation of the manner of pairing of the inverted homologues of 25c.

25e Subterminal inversion of "C" chromosome found in different stock from that from which 25a, b, and c drawings were made.

26 "X" chromosome.

27a End 1 of "B" chromosome which shows a small inversion.

27b Diagrammatic representation of inversion shown in 27a and 28a.

27c End 2 of "B" chromosome.

28a End 1 of "A" chromosome. Great deal of asynapsis is due to inversion which makes each homologue loop at right angles to its longitudinal axis, as well as to band differences.

28b End 2 of "A" chromosome. Asynapsis shown here in some regions without band difference. Arrow points to a terminal difference in which one homologue is deficient for a small amount of material.

29–32 *Sciara nacta.* Salivary gland chromosomes.

29a End 1 of "C" chromosome.

29b End 2 of "C" chromosome with characteristic terminal puff.

29c Short subterminal inversion of "C" chromosome in a second stock of *S. nacta.*

29d Short subterminal inversion of "C" chromosome with vesicular band terminal deletion, marked by the arrow, found in a third stock of *S. nacta.*

30a End 1 of "B" chromosome. Arrow indicates a small band difference wherein one homologue contains a compound band and the other homologue at the corresponding locus only a single band.

30b End 2 of the "B" chromosome.

31a End 1 of the "A" chromosome.

31b End 2 of the "A" chromosome.

32a End 1 of the "X" chromosome showing the characteristic bifurcated tip and the knotted loose figure eight caused by the synapsis of three apparently homologous regions. (Double repeat).

32b End 2 of the "X" chromosome.

EXPLANATION OF FIGURES, PLATE V

33–36 *Sciara fenestralis*, form II. Salivary gland chromosomes.

33a End 1 of "A" chromosome.

33b Short intercalary piece of "A" chromosome in which arrow points to solid band apposed to a vesicle.

33c End 2 of "A" chromosome. Arrow indicates a short homogeneous region opposite a banded region.

33d Short intercalary inversion of "A" chromosome.

34 Inverted "B" chromosome with short included inversion.

35a End 1 of "X" chromosome.

35b End 2 of "X" chromosome.

36a Synapsed ends of inverted "C" chromosome. Arrow indicates thin band apposed to thick band.

36b Large "C" chromosome inversion. Arrow indicates a vesicle apposed to a double band.

37–40 *Sciara fenestralis*, form I. Salivary gland chromosomes.

37a Duplicate of end of "X" chromosome, shown beneath it, in which banding pattern is better.

37b Repeat loop of "X" chromosome. Repeated bands synapsed to form loop.

37c Other end of "X" chromosome.

38a Long subterminal inversion of "C" chromosome.

38b A different view of the ends shown in 38a in which banding pattern of the two non-synapsed ends is good.

39a End 1 of "A" chromosome.

39b End 2 of "A" chromosome. Arrow points to "small difference" in which a thin band is apposed to a thick band.

40a End 1 of "B" chromosome.

40b End 2 of "B" chromosome.

Explanation of Figures, Plate VI

41–44, 46–48 Photographed at magnification indicated above Figure 1. All photographs were made from the dorsal surface of the right wing of the male.

41 *Sciara nacta.*

42 *Sciara agraria.*

43 *Sciara species XXV.*

44 *Sciara fenestralis*, form II.

45 *Sciara prolifica* (Felt), Johannsen's var. b. Magnification scale indicated beneath it.

46 *Sciara species XXIII.*

47 *Sciara species VII.*

48 *Sciara fenestralis*, form I.

Explanation of Figures, Plate VII

Magnification scale drawn beneath each figure represents .1 mm. Each figure represents the ventral surface of a male hypopygium.

49 *Sciara species VII.*

50 *Sciara nacta.*

51 *Sciara prolifica* (Felt), Johannsen's var. b.

52 *Sciara species XXV.*

53 *Sciara fenestralis*, form II.

54 *Sciara fenestralis*, form I.

55 *Sciara species XXIII.*

56 *Sciara agraria.*

PLATE IV

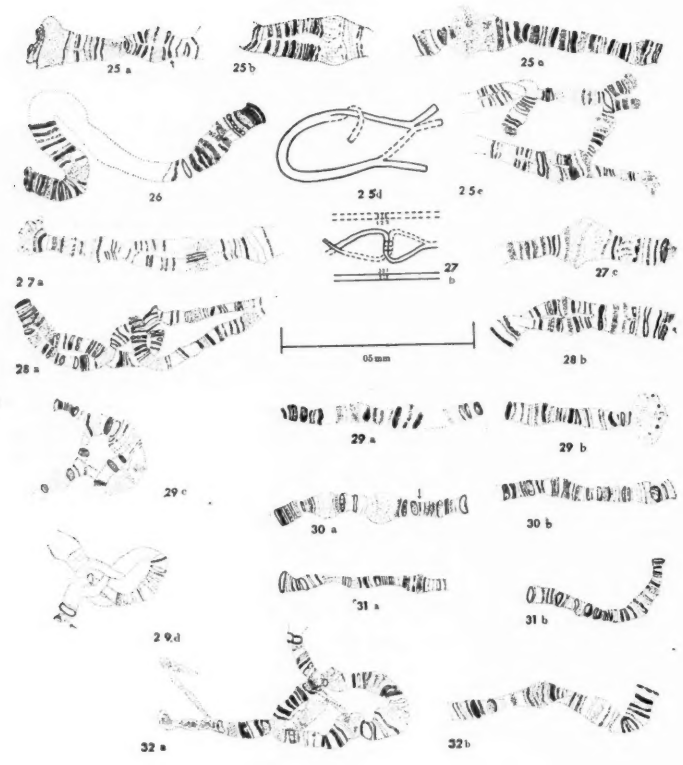

25 a
25 b
25 c
26
25 d
25 e
27 a
27
b
27 c
28 a
0·5 mm
28 b
29 a
29 b
29 c
30 a
30 b
29 d
31 a
31 b
32 a
32 b

PLATE V

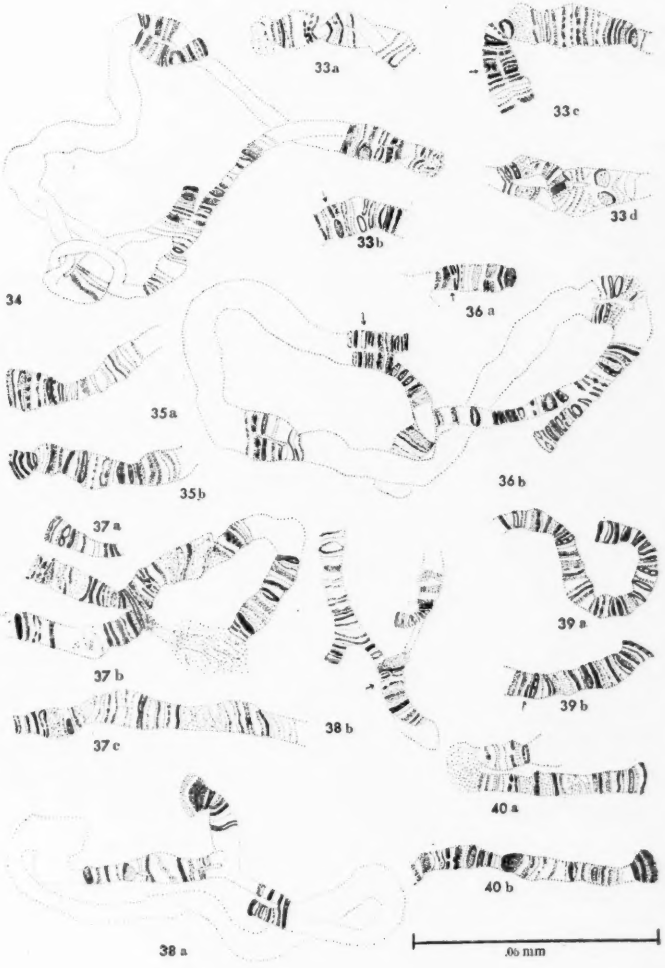

33a
33c
33d
33b
34
36a
35a
35b
36b
37a
37b
37c
38b
39a
39b
40a
40b
38a
.05 mm

PLATE VI

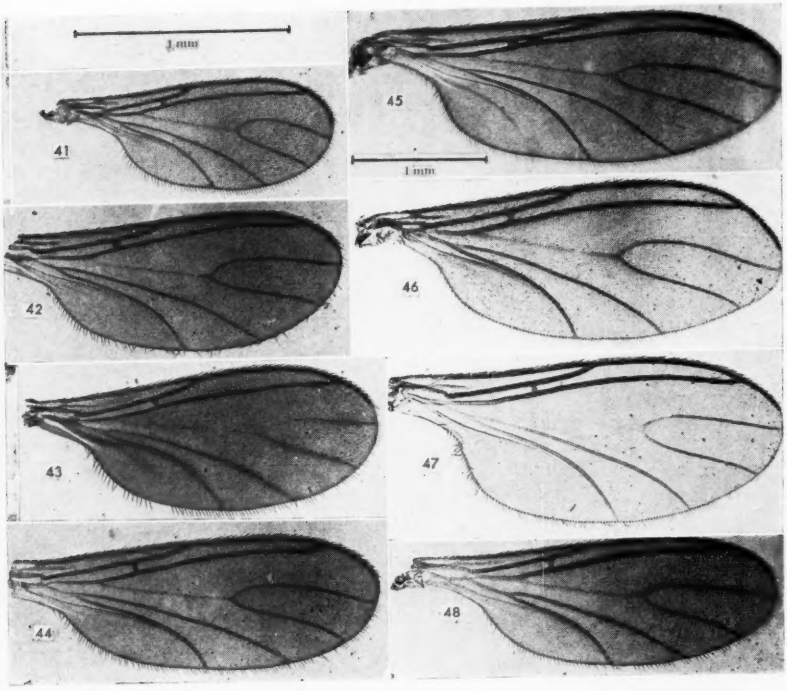
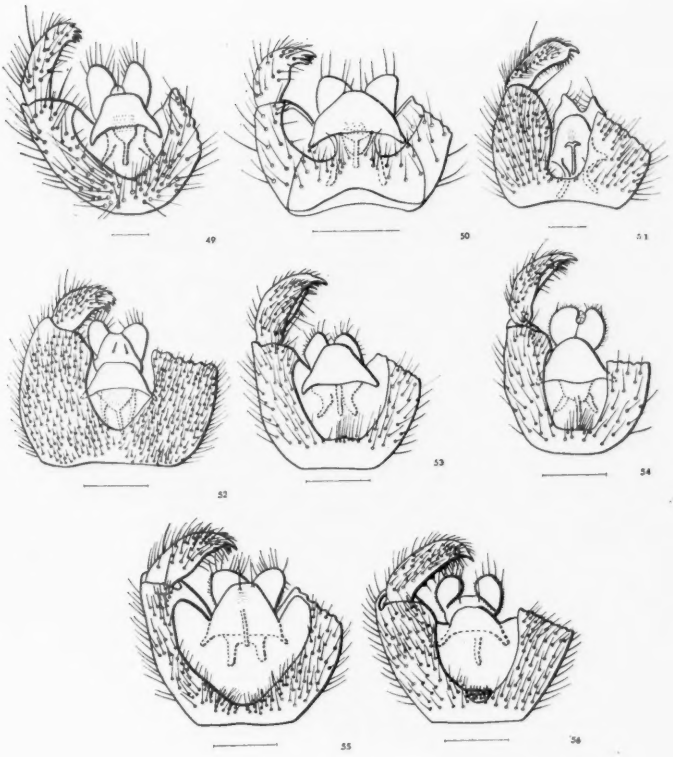


PLATE VII



PHENOGENETIC STUDIES OF THE HOMOEOTIC MUTANTS OF *DROSOPHILA MELANOGASTER*

III. THE EFFECTS OF TEMPERATURE ON THE EXPRESSION OF BITHORAX-34E

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THE mutant bithorax (chromosome 3, locus 58.8, symbol bx), the first homoeotic mutant of *Drosophila melanogaster* to be discovered, was described by Bridges and Morgan (1923). The phenotype of bithorax is very variable and overlaps the wild type. In flies which show the bithorax condition the metathoracic segment resembles the normal mesothoracic segment: an extra thorax and scutellum appear as an outpushing covered with hairs and bristles between the normal mesothoracic scutellum and the abdomen; the halteres are enlarged and either inflated and vesiculate or flattened and wing-like, with typical venation and large costal bristles. Bridges stated that in particularly favorable specimens the bristles which appear on this metathoracic outgrowth can be homologized exactly with the normal mesothoracic bristles, the dorso-centrals, post-alars, supra-alars, etc. Six other alleles of bx have since been found which vary in their dominance, viability and degree of expression. In this study the allele bx-34e, found by Schultz in 1934, was used because its expression is higher and more constant than the original bx.

A review of the phenomenon of homoeosis and its bearing on questions of comparative anatomy, embryology and evolution has been given elsewhere (Goldschmidt, 1940; Villee, 1942c). From the data on the development of the antennal discs in normal and aristapedia flies presented by Balkashina (1929), Goldschmidt (1938, 1940) derived a hypothesis of the action of homoeotic loci in producing their effects. He postulated that all the imaginal discs have identical potencies very early

in development. Each becomes mature (or "competent" (Waddington)) at a certain stage in development different from the others. A series of evocators, one for each type of disc, is given off by the germ, each at the proper time to affect the proper disc which has just become mature and cause it to develop in the normal way. The homoeotic mutants upset this timetable so that a particular disc becomes affected by an evocator other than its normal one and so is differentiated into a structure abnormal for that location, *e.g.*, an antennal disc develops as a leg, or the labial bud develops as a leg or an arista. Balkashina found that the normal antennal disc begins to segment in the 4- or 4½-day larva, whereas the aristapedia antennal disc begins to segment in the 2-day larva, soon after the leg discs begin to segment. According to Goldschmidt, sometime during or before the second day of larval life an evocator is given off which diffuses into the six leg discs and causes a rhythmic subdivision of the distal end of the anlage, producing normal tarsus segmentation. Normally only leg discs are competent at this time and react to this induction, but any disc which happens to be competent will react by tarsus formation. The aristapedia gene, therefore, speeds up the maturation of the antennal disc, and causes it to become competent in the 2-day rather than the 4-day larva, so that the tarsus evocator present in the 2-day larva may induce it to form a tarsus.

Astauroff (1930) and Villee (1942b) reported the marked effect of extreme temperatures during development on the penetrance of tetraptera and tetraltera, respectively. In tetraptera, a homoeotic mutant resembling bithorax, there was a direct relationship between temperature and penetrance, the penetrance being about 1 per cent. at 17° C. and about 35 per cent. at 25° C. In tetraltera, in which the wings are changed to halteres, there was an inverse relationship between penetrance and temperature, the penetrance varying from less than 1 per cent. at 29° C. to over 35 per cent. at 14.4° C. Other studies on aristapedia and aristapedia-Bridges (Villee,

1942a, 1943a, b) and proboscipedia (Villee, 1943d, 1944) revealed that heat treatments (29° C. and 35° C.) given at various periods of development shifted development of the antenna of aristapedia flies toward the arista phenotype, while cold treatments (14.4° C.) shifted development toward the tarsus phenotype, whereas heat treatments shifted the development of the labial buds of proboscipedia flies toward the tarsus phenotype and cold treatments shifted development toward the arista phenotype. The present study was undertaken to determine the effects of heat and cold treatments on the development of the metathoracic discs of bithorax larvae. A preliminary report was given previously (Villee, 1943c).

MATERIALS AND METHODS

A study of the phenotypes of flies from the bx-34e stock and from preliminary heat and cold experiments showed that a graded series occurred ranging from a normal haltere (called type 1) to a large, flat, wing-shaped structure complete with four longitudinal veins (type 7). The series is practically identical with that described by Astauroff (1929) for tetraptera. Type 2 resembles a haltere, but the distal segment (head) is enlarged and bears a row of costal bristles. In type 3 the head is larger and beginning to flatten in the middle. Type 4 is larger and flatter than the preceding and has more bristles and hairs. Type 5 is large, flat and wing-like but has no wing veins. Type 6 is again larger and shows one or two longitudinal veins while type 7 has four longitudinal veins. The metathoracic wing is never more than about half the size of the normal mesothoracic wing.

Large numbers of male and female bx-34e flies were placed in half-pint culture bottles containing the usual cornmeal-agar-molasses medium and left for 2-hour egg-laying periods. The parents were then removed and the cultures kept at 22° C. until the temperature treatments were applied. After the treatments, the cultures were returned to 22° C. until the imagos hatched.

a. Effect of heat treatments on the development of bithorax-34e. To test the effect of high temperatures during development, groups of three cultures each were prepared as described above and placed in an incubator regulated at 29° C. beginning 0, 1, 2, 3, 4 or 5 days after the parents were removed and continuing 1 to 6 days. A

TABLE 1

EFFECT OF 29° C. TREATMENTS ON THE EXPRESSION OF BITHORAX-34E

Days of development Treatment		Bithorax types						Total	Code
Begun	Ended	1	2	3	4	5	6		
0	1	..	7	13	..	..	..	20	265
0	2	..	17	89	25	..	..	131	306
0	3	..	1	45	3	..	..	49	304
0	4	1	6	80	23	..	..	110	314
0	5	..	6	24	10	..	..	40	310
0	6	..	2	31	7	..	..	40	312
1	2	1	53	141	2	..	..	197	272
1	3	1	43	209	3	..	..	256	284
1	4	..	4	103	49	1	..	157	330
1	5	1	9	133	50	..	..	193	320
1	6	..	5	77	13	..	..	95	309
1	7	1	9	132	19	..	..	161	305
2	3	..	42	92	6	..	..	140	274
2	4	1	8	119	40	..	..	168	317
2	5	..	6	60	24	..	..	90	320
2	6	..	7	95	31	1	..	134	320
2	7	..	1	31	18	..	..	50	334
2	8	..	..	116	38	..	..	154	325
3	4	..	38	110	..	..	..	148	274
3	5	..	33	150	17	..	..	200	291
3	6	..	..	3	1	..	..	4	325
3	7	..	..	7	2	..	..	9	322
3	8	..	..	9	3	..	..	12	330
3	9	..	..	6	4	..	..	10	340
4	5	..	10	8	2	..	..	20	260
4	6	..	6	62	44	..	..	112	334
4	7	..	1	76	36	..	1	114	334
4	8	..	2	43	5	..	..	50	306
4	9	..	..	9	3	..	..	12	325
4	10	..	..	2	8	..	..	10	380
5	6	..	1	6	..	..	..	7	286
5	8	..	..	5	..	..	..	5	300
5	9	..	..	6	6	..	..	12	350
							Grand total	2,910	

total of thirty-three groups of three bottles each were prepared and given heat treatments of different durations begun at various times after the eggs were deposited. Following the treatment the cultures were returned to 22° C. Six cultures were kept at 22° C. as controls and another group of eight cultures were kept at 29° C. throughout development. The adult flies which

hatched from the treated larvae were classified as to type of metathoracic appendage and counted. The results of the counts of the three bottles in each group were combined and are presented in Table 1. To simplify comparisons between treatments, the system of code numbers devised earlier (Villee, 1943b) was used. The percentage of flies of each type present in each of the experimental groups was calculated. The code number is the sum of the products of the percentage of flies of each type times

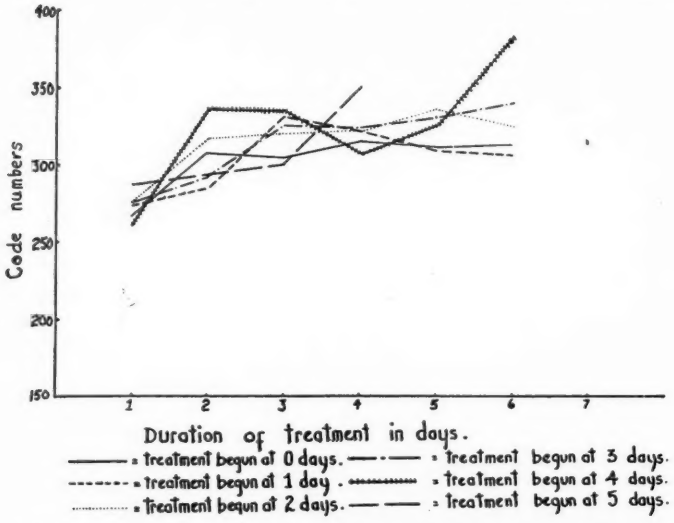

FIG. 1. Variation in the effect of heat treatments on the expression of bithorax-34e (given in code numbers) with the duration of the treatments. Each curve represents treatments begun on a particular day of development.

the respective type number (1 to 7). The code number, therefore, lies between 100 (if 100 per cent. of the group were type 1) and 700 (if 100 per cent. of the group were type 7). By this means a single number represents the effect of a particular treatment on the development of the metathoracic appendage in bithorax. The mean code number of the control group kept at 22° C. throughout development and its standard deviation was 267 ± 6.5. The mean code number and standard deviation of the group kept at 29° C. throughout development was 302 ± 10.5.

In Fig. 1 these code numbers have been plotted against the duration of the treatments in days to present graphically a comparison of the effectiveness of the various treatments in increasing the expression of the mutant

TABLE 2

EFFECT OF 15° C. TREATMENTS ON THE EXPRESSION OF BITHORAX-34E

Days of development Treatment		Bithorax types				Total	Code
Begun	Ended	1	2	3	4		
0	1	..	..	16	..	16	300
0	2	..	11	88	..	99	289
0	3	..	27	101	..	128	279
0	4	..	77	126	..	203	262
0	5	1	74	60	..	135	243
0	6	..	92	34	..	126	227
0	7	..	54	29	..	83	235
1	2	..	13	74	..	87	285
1	3	..	8	67	3	78	294
1	4	..	86	58	..	144	240
1	5	..	64	78	..	142	255
1	6	..	77	31	..	108	229
1	7	..	33	15	..	48	231
2	3	..	12	40	2	54	282
2	4	..	3	33	..	36	292
2	5	..	43	66	..	109	261
2	6	..	22	13	..	35	237
2	7	2	67	37	..	106	233
2	8	24	29	2	..	55	159
3	4	..	13	88	1	102	288
3	5	..	43	97	2	142	272
3	6	..	17	31	1	49	267
3	7	..	60	61	4	125	255
3	8	..	15	19	..	34	256
3	9	1	4	..	..	5	180
3	10	4	45	1	..	50	194
3	11	2	16	..	..	18	189
4	5	..	14	9	..	23	239
4	6	..	6	5	..	11	245
4	7	1	49	41	1	92	243
4	8	..	41	15	..	56	227
4	9	1	17	3	..	21	209
4	10	5	26	..	..	31	184
4	11	1	7	1	..	9	178
4	12	2	21	..	..	23	191
5	6	..	..	18	3	21	314
5	7	..	42	56	..	98	257
5	8	2	81	41	..	124	232
5	9	..	106	30	..	136	222
5	11	1	39	13	..	53	223
5	13	2	57	6	..	65	206
					Grand total	3,080	

(the greater the transformation of the haltere into a wing, the higher the code number). The increase in expression is roughly proportional to the length of the treatment up to a maximum of three or four days, after which a continuation of the treatment has little or no effect in

increasing the expression. Treatments begun four days after egg deposition were slightly more effective in increasing expression, *i.e.*, in causing a shift in development toward a wing rather than a haltere, than those begun earlier or later. The code number of the control group, 267 ± 6.5, may be used in judging the effectiveness of the several treatments.

b. Effect of cold treatments on the development of bithorax-34e. Cultures of bx-34e eggs were prepared as

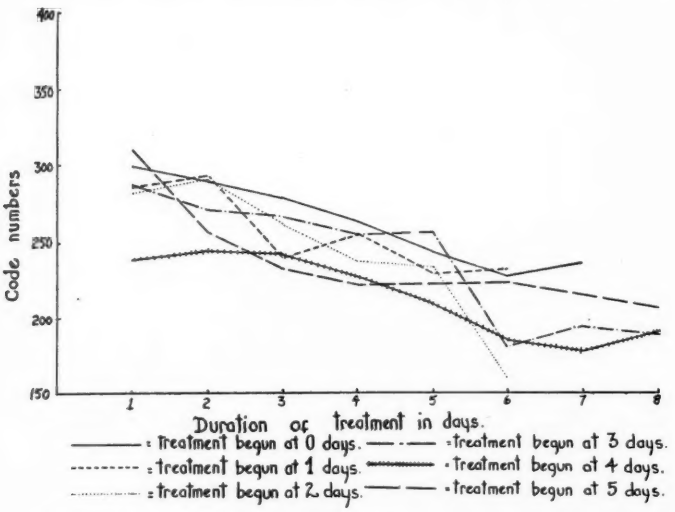

FIG. 2. Variation of the effect of cold treatments on the expression of bithorax-34e (given in code numbers) with the duration of the treatments. Each curve represents treatments begun on a particular day of development.

described above and placed in groups of three in an incubator regulated at 15° C. beginning 0 to 5 days after egg deposition and continuing 1 to 8 days. A total of forty-one groups of three bottles each were prepared and subjected to low temperatures during various periods of development. After the treatments the cultures were returned to 22° C. to complete development. The adult flies which hatched were classified as to metathoracic appendage and counted. The results of the counts of the

three cultures comprising each group were combined and are given in Table 2 together with the code numbers derived from the figures. These code numbers were then plotted against the duration of the treatments in days (Fig. 2) to show graphically the relative effects of the different treatments in decreasing the expression of bithorax (the less the haltere has been transformed into a wing, the lower the code number). The decrease in expression again is roughly proportional to the duration of the treatment. Treatments begun four days after the eggs were laid were most effective in decreasing the expression, those begun three or five days were less effective and those begun at other times were still less effective in decreasing the expression of bithorax, *i.e.*, in causing a shift in development toward a haltere instead of a wing. The code number of the control group, 267 ± 6.5, may again be used as a base line in judging the effectiveness of the several treatments.

Discussion

The finding that high temperatures increase the expression of bx-34e, causing an increase in the shift in development toward the wing phenotype, and low temperatures decrease this, causing development to shift toward the haltere phenotype agrees with the results of Astauroff's (1930) experiments with tetraptera and Villee's (1942b) work with tetraltera. In all three, high temperatures shift development of the meso- or metathoracic disc toward the wing phenotype and low temperatures shift development toward the haltere phenotype. Since the embryological processes resulting in the bithorax or tetraptera phenotype, the changing of a haltere anlage to produce a wing, are presumably opposite to those resulting in the tetraltera phenotype, the changing of the wing anlage to produce a haltere, the opposite relationship between temperature and penetrance or expression in the stocks is indicative. The action of temperature on development in general is to accelerate or retard differentially certain processes in relation to others, since the chemical

reactions involved will probably have different temperature coefficients. Villee (1942b, c) assumed, therefore, that the production of a wing instead of a haltere requires the differential acceleration of some process or processes which is increased by higher temperatures during or before the determination and differentiation of the wing and haltere discs; whereas the production of a haltere instead of a wing requires the differential retardation of these same or similar processes which is increased by lower temperature. These data do not enable us to tell what part or parts of the developmental system the temperature treatments are affecting. They may be affecting the rate of maturation of the meso- or metathoracic discs, the rate of production of the evocator substance, or something else. The study of proboscipedia (Villee, 1944) showed that the effects of temperature treatments were best explained by supposing that they affect the rate of maturation of the imaginal discs and probably the same holds for bithorax.

Chen (1929) and Auerbach (1936) found that the wing bud develops from the central part of the dorsal mesothoracic disc during the latter part of the fourth day of larval development and that the first five prepupal hours are critical for wing formation. The wing everts during the sixth prepupal hour. The haltere bud is recognizable at the end of the larval period (4–4½ days) and is completely formed and segments during the first five prepupal hours. Robertson (1936) found that the haltere bud evaginates a little later than the wing bud. We may therefore assume that the mesothoracic evocator is released before the metathoracic evocator. If the reverse should later be found to be true, the following hypothesis would not be invalidated but could still be true after minor changes in the details.

We may now apply the theory suggested by Goldschmidt (1938, 1940) outlined previously and the modifications suggested by Villee (1944) to explain the action of tetraltera, tetraptera and bithorax and the effects of

temperature on their development. If we assume that in the normal, wild-type fly, each disc matures at the proper time to be affected by the normal evocator for that disc, we may call this rate of maturation 1. The dorsal mesothoracic disc normally becomes competent when the mesothoracic evocator is released and responds to the induction by the formation of a wing. The metathoracic disc normally becomes competent a little later, when the metathoracic evocator is released, and responds by the

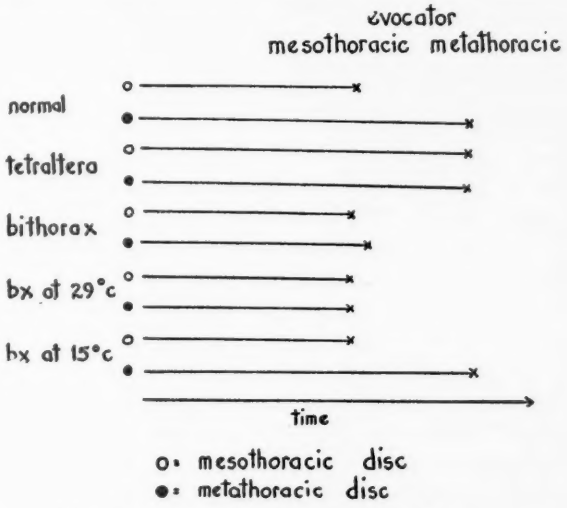

FIG. 3. Scheme of the interactions of maturation and evocator release to produce normal or abnormal induction of the dorsal meso- and metathoracic discs. See text for discussion.

formation of a haltere (Fig. 3). If we now assume that the bithorax and tetraptera genes alter the rate of maturation of the metathoracic disc so that it is greater than 1, the metathoracic disc will become competent faster, hence earlier, and will be affected by the mesothoracic evocator and respond by the formation of a wing and a bristle-covered structure resembling a scutellum and mesonotum. Similarly the tetraltera gene may alter the rate of maturation of the mesothoracic disc so that it is

less than 1 (less than its normal rate, whatever that may be), so that the mesothoracic disc becomes competent later than normal, is affected by the metathoracic evocator and responds by the formation of a haltere and a metathoracic-like dorsal mesothorax.

The temperature treatments affect this system by accelerating or decelerating the rate of maturation of the discs differentially from the rate of development as a whole. In bithorax, we assume that the mutant gene changes the rate of maturation of the metathoracic disc, speeding it so that it becomes competent earlier in development than normal. High temperatures applied during development will speed all chemical reactions but will speed those leading to the maturation of the metathoracic disc more than the others because the bx gene has altered the rate of development of the metathoracic disc relative to the rate of development as a whole. This additional effect of temperature will thus cause the metathoracic disc to become competent even earlier and be affected more strongly by the mesothoracic evocator, and causing a greater shift towards the wing phenotype than at normal temperatures. Conversely, low temperatures applied during development will slow down all chemical reactions but will slow those leading to the maturation of the metathoracic discs more than the others. The temperature treatments will thus cause the metathoracic disc to become competent relatively later in development; in extreme cases, so late that it is not affected by the mesothoracic evocator but by the metathoracic one. Similar considerations explain the effect of temperature on the penetrance of tetraptera and tetraltera.

It is unnecessary for this hypothesis that the evocators be diffusible substances produced in one part of the body (Goldschmidt's "germ") and diffusing into the imaginal discs, resembling the eye color hormones which are produced in the fat bodies and Malpighian tubules and diffuse into the eye discs. The evocators may be substances of a molecular size too large to diffuse through cell mem-

branes which are produced in the nucleus of each cell of an imaginal disc (as well as in all other cells of the body) by some particular locus, and released to the cytoplasm when the nuclear membrane breaks at mitosis.

The fact that bithorax affects the whole metathoracic disc as well as the haltere bud has been noted. The metanotum is changed into a structure resembling a mesonotum and scutellum on which are bristles similar to those on the normal structures. The temperature treatments affected the development of these structures as well: low temperatures during development caused them to be very small or absent, high temperatures during development caused them to be large and covered with many bristles. An extension of the explanation offered above would cover this phenomenon as well.

The phenomenon investigated here is an example of the "branching-track" sort of development discussed by Waddington (1940). The branching is not so definite as in aristapedia or proboscipedia, where the intermediate forms have one part definitely arista-like and another tarsus-like with a sharp transition line between. In bithorax the intermediates have some wing and some haltere characteristics, with a complete series of intergrades between wing and haltere. These intermediates may indicate that the metathoracic evocator is released shortly after the mesothoracic evocator so that intermediates are easily produced, whereas tarsus and arista evocators are given off at quite different times in development so that induction occurs one way or the other, with a sharp line of transition between. In terms of Waddington's "epigenetic landscape," the valleys are not so deep nor the cliffs so high in the branching between wing and haltere as between tarsus and arista so that development may fail to go in one valley or the other but may go somewhere between. Other possible explanations involve thresholds which differ progressively along the length of the disc in aristapedia but which are absent in bithorax, or some essential difference in the ability of the imaginal discs to react.

SUMMARY

Bithorax-34e flies were given high and low temperature treatments during development, beginning 0 to 5 days after the eggs were laid and continuing 1 to 8 days. Heat treatments increased the expression of the mutant, causing an increase in the change of the haltere to a wing. Cold treatments decreased the expression, causing the metathoracic appendage to be more haltere-like. The increase or decrease was roughly proportional to the duration of the treatment and treatments begun four days after egg deposition were slightly more effective in shifting development than those begun earlier or later. The treatments affected the development of the extra thorax and scutellum which appear between the normal scutellum and abdomen in a similar fashion. The application of Goldschmidt's theory of the action of homoeotic loci to these data is discussed.

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PROLONGATION OF LARVAL–PUPAL DEVELOPMENT IN *DROSOPHILA MELANOGASTER* AND ITS EFFECT ON FACET NUMBER[1]

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INTRODUCTION

THERE are several instances in the literature in which the effect of prolongation of developmental time and the resultant effect on gene expression have been reported. Only a few of these reports were concerned with facet number in *Drosophila melanogaster*. Braun (1939) and Green and Oliver (1943) investigated the effect of prolongation of development in the larval stage on the expression of genes for wing characters in *Drosophila melanogaster*. They postulated the elaboration of a substance by the factors involved. Prolongation of development enhanced the expression of the responsible factors by allowing additional time for elaboration of the substances which resorb the already fully formed material that would normally form the character as in the case of notched wing as studied by Braun. Green and Oliver, in brief, report on experiments with vestigial wing in which they found that prolongation of developmental processes resulted in an increased expression of the vestigial character due to an increase in the amount of substance elaborated by the factor responsible. Luce (1939, 1940, 1942) studied the effects of formalin, of oxygen and of a mixture of oxygen and carbon dioxide on facet number in bar alleles of *Drosophila melanogaster*. He found (1942) that high concentrations of formalin increased the length of development and reduced the mean facet number. In this instance the lengthening of development enhanced the expression of the bar factors. The oxygen studies of

[1] A part of a thesis entitled "The Physiology of the Development of the Imaginal Buds in the Bar Series of *Drosophila melanogaster*" submitted for the degree of doctor of philosophy in zoology in the Graduate School of the University of Illinois, 1944.

the same author conducted on a bar infrabar strain (1940) produced different results. The time of development was prolonged, but the expression of the gene difference at the bar locus was decreased as the facet number of the flies was greater than in the controls. His studies on the effect of a mixture of 95 per cent. oxygen and 5 per cent. carbon dioxide (1939) yielded similar results in an infrabar strain.

VITAMIN B_1 (THIAMIN HYDROCHLORIDE)

The present study was carried out upon bar and ultrabar strains of *Drosophila melanogaster* and dietary deficiencies were utilized to induce prolongation of development. Schader (1941) noted a prolongation of larval development due to a diet deficient in vitamin B_1. Keenan, Kline, Elvehjem and Hart (1935) devised a technique for inactivating vitamin B_1 in fresh cake yeast. Van't Hoog (1935) formulated a synthetic culture medium for insects which could be prepared and kept sterile. Baumberger (1919) found that microorganisms were a source of nutrition to insects. He also showed that autoclaved yeast was a completely adequate food for *Drosophila melanogaster*.

VITAMIN E

There is no conclusive evidence that insects need any other vitamin than vitamin B for normal development. Hill and Burdett (1932) believed, however, that the royal jelly of honey bees, which is the substance necessary for the transformation of worker larva to the queen or sexually mature form, was a rich source of vitamin E. They interpreted their results as indicating the presence of appreciable amounts of the vitamin in royal jelly, but subsequent investigations by Mason and Melampy (1936), Evans, Emerson and Ackert (1937), and Haydak and Palmer (1939) failed, after careful assay, to reveal any vitamin E in this substance. The utilization of vitamin E inactivation methods (Waddell and Steenbock, 1928) were

employed on the culture medium used in part of this study to determine if this vitamin is essential in *Drosophila* growth and development.

Material and Methods

The strains of flies, bar and ultrabar, used in this experiment had a sufficiently reduced number of facets so that the "error factor" in counting facets was greatly minimized. These strains were originally derived from a bar-eyed line inbred since 1923, at a constant temperature of 27° C. This is the so-called "temperature-inheritance line" of the University of Illinois.

The culture medium used for the stock flies from which individuals were obtained for the diverse experiments is a modification of the Russian formula by Luce (unpublished). It comprises these ingredients:

80 ml biological water (chlorine free)
1.5 gm agar agar
1.5 " dried brewer's yeast
6.0 " yellow corn meal
6.0 " syrup
5.0 " ground raisins
a small amount of powdered yeast foam to each vial

The water, agar agar and corn meal were heated and allowed to boil until the ingredients were almost cooked. The brewer's yeast, syrup and raisins were then added, and all were permitted to boil for several minutes. The food was then placed in 25 × 100 mm shell vials, about one inch of food to each vial. Powdered yeast foam was sprinkled lightly on the food surface after it had cooled and solidified.

Vitamin E Deficiency

The vitamin E inactivated medium was obtained by treating the corn meal and raisins of the medium with ferric chloride and ether (Waddell and Steenbock, 1935). One per cent. by weight of ferric chloride, which had been powdered, mixed with a quantity of ether equal to the volume of the food treated was the mixture used for in-

activation of the vitamin E contained in the food. The oxidizing medium and the food treated were kept in a closed vessel for 24 hours to permit extraction of the fat soluble vitamin and the oxidation of it. After 24 hours, the ether was allowed to evaporate by removing the cover of the vessel. After complete evaporation of the ether, the treated food was ready for use in the preparation of the experimental medium. To check on the possibility of lethal effects or toxicity due to the presence of the ferric chloride, a second medium was prepared which contained an amount of ferric chloride equal to that used in the inactivation process.

VITAMIN B_1 DEFICIENCY

A vitamin B_1 deficient medium was prepared synthetically (Van't Hoog, 1935). It contained agar agar, sucrose, yeast and Pearl's salt mixture, modified by Sang. The salt mixture consisted of three different solutions:

SOLUTION A

8.00	gm	potassium sodium tartrate
2.00	"	ammonium sulfate
0.50	"	magnesium sulfate
250.00	ml	distilled water

SOLUTION B

5.00	gm	tartaric acid
0.65	"	potassium dihydrogen phosphate
250.00	ml	distilled water

SOLUTION C

0.25	gm	calcium chloride
250.00	ml	distilled water

The solutions were autoclaved for 20 minutes at 250° F. and under 15 pounds of steam pressure. In the preparation of the experimental medium, 10 grams of Fleischmann's fresh cake yeast was added to a flask containing 25 ml of distilled water. This was autoclaved for 5 hours at 250° F. and under 15 pounds of steam pressure. This treatment inactivated the thiamin hydrochloride present in the yeast (Keenan, Kline, Elvehjem and Hart, 1935).

Schader (1941) and others have found that this inactivation process also affects the potency of riboflavin and nicotinic acid present in the yeast. Because of this it was necessary to add these vitamin B components in the proportion of 14.3 mg of nicotinic acid and 0.05 mg of riboflavin to each 100 ml of the culture medium. The autoclaved yeast was added to 25 ml of solution A and 25 ml of solution C. The nicotinic acid, riboflavin, 1.5 grams of agar agar and 1 gram of sucrose were added to this mixture. Twenty-five ml of solution B were contained in another flask. The two flasks were autoclaved for 20 minutes at 250° F. and under 15 pounds of steam pressure. Solution B was then mixed with the contents of the other flask and the resultant mixture was boiled for one minute. Three ml of this mixture were transferred to sterile cotton-stoppered, 25 × 100 mm shell vials by means of sterile pipettes. The food was allowed to cool and solidify. It was important that the steps as outlined be followed, since hydrolization of the agar agar occurred if this precaution was not observed. In preparing the control and check media, the same procedure was observed except that in the control the yeast was not autoclaved for more than twenty minutes. Also the riboflavin and nicotinic acid were not added. In a check medium experiment the culture medium consisted of the experimental medium plus an amount of thiamin considered to be equivalent to that inactivated. The thiamin added was contained in a sterile aqueous solution. The amount added was 0.08 mg/100 ml of culture medium.

Parent Flies and Egg Collecting

The stocks of flies used in the study had been closely inbred at a constant temperature of 24° C. ± 0.5°. From these stocks pure male and female ultrabar and bar flies were selected. Eight males and 8 females were placed in each vial containing the stock culture medium. They were inbred for several more generations and then used for this study.

The method of collecting eggs is original and permits of securing large numbers in a short time. It also facilitates handling of eggs in transferring them to vials. Strips of absorbent paper-toweling were cut to fit into the shell vials. Onto these paper strips a 1 per cent. agar agar solution was dropped in the shape of oval buttons. After the agar had solidified, the "buttons" were grooved with a needle, painted with a solution of 2 parts of glacial acetic acid, 10 parts sorghum molasses and 88 parts distilled water. The paper strips were then transferred to the shell vials containing 8 pairs of the stock flies to be used for parents. The vials containing the strips of paper were placed in a lidded, metal chamber lined with moistened absorbent paper. Thus all the factors conducive to greater egg production were supplied. The ovipositing period was of four hours' duration and the age of the egg-larva could be determined with a – 2 hour error if the midpoint of the egg-laying period was used as the time of oviposition (Alpatov, 1929).

To collect the eggs the agar "buttons" were placed in a Petri dish after removal from the paper strips. They were observed under a binocular microscope and a determination of the number of eggs on each "button" was made. The eggs were rendered aseptic as follows. A sufficient volume of 85 per cent. alcohol was poured into the Petri dish to cover the "buttons." This treatment destroys any yeast cells which might be clinging to the eggs but does not injure the embryo within (Schader, 1941). According to Baumberger (1919), yeast cells are not transmitted from the adult female to the eggs. This procedure greatly obviated the possibility of contaminating the culture media used in these experiments. After one hour in 85 per cent. alcohol, the agar "buttons" were transferred to vials with a sterile dissecting needle.

Temperature Control, Asepsis and Calculations

The experiments of the present study were all carried out at the optimum temperature for *Drosophila melano-*

gaster development, *i.e.*, 24° C. ± 0.05°. This was accomplished by the use of a constant-temperature chamber specially constructed and well insulated containing both cooling and heating units under automatic control. A thermograph made constant recordings of the temperature.

Aseptic conditions were maintained throughout the study by autoclaving all cultural media, food vials, pipettes, etc. The vials used for flies were flamed whenever transfers were made, the eggs were placed in 85 per cent. alcohol for one hour, and all contaminated vials were immediately discarded.

Hourly observations were made during the critical developmental periods and a record of time spent in larval and pupal development was obtained. The facets of the compound eyes of random samples of flies obtained from these experiments were counted under an E. Leitz, Ultrapak microscope. The random sample consisted of 20 to 40 individuals of each sex for each culture medium used. Statistical constants were obtained from these samples.

Experimental Results

The experimental results are presented in tabular form comparing length of developmental times, the mean of the facet number for males and females, standard deviation of the mean and standard error of the mean. Statistical significance is also indicated in these tables.

In all the experiments conducted the rate of mortality, the fertility and the sex ratio of the flies were essentially normal.

Discussion

The data obtained from this study are significant in several respects. From a nutritional viewpoint it is evident that thiamin hydrochloride is not necessary to complete development though it is necessary for normal development. This would be in accord with the results of Lafon's findings (1935) and Schader's (1941). A study

of Table 1 would indicate that the amount of thiamin added to the culture medium which had been subjected to vitamin B_1 inactivation was not equivalent to the amount inactivated, for we find an extension of the developmental periods where this medium is employed as well as a significant increase in facet number. It can be concluded that this medium was partly deficient in thiamin, which

TABLE 1

PROLONGATION OF LARVAL AND PUPAL STAGES OF DEVELOPMENT DUE TO VITAMIN B_1 DEFICIENCY AND A CONSEQUENT INCREASE IN FACET NUMBER IN A BAR STRAIN

		I Control	II Experimental organisms cultured on B_1 inactivated food to which .08 mg of B_1/100 ml of culture medium was added	III Experimental organisms cultured on a medium treated for inactivation of Vitamin B_1
Mean larval period in hours		190	202	323
Mean pupal period in hours		110	120.5	167
Facet number	Males	132.10	150.45	162.28
	Females	83.10	87.31	95.00
Standard deviation of the mean	Males	± 5.27	± 3.67	± 4.75
	Females	± 6.32	± 2.92	± 4.55
Standard error of the mean	Males	± .96	± .62	± .91
	Females	± 1.38	± .61	± .91
Statistical significance	Males	+ with II	+ with III	+ with I
	Females	+ with II	+ with III	+ with I

(+ = statistical significance.)

resulted in a reduced effect on both lengthening of development and facet number as compared to complete inactivation of thiamin in the other experimental medium.

It is not presumed that the inactivation of vitamin E was the causative factor in prolonging development in the ultrabar strain studied. The report of Tatum (1939) and some unpublished work by the author indicates that no other vitamin than vitamin B is essential to *Drosophila* development. It is evident that the addition of ferric

chloride to the culture medium had no significant toxic effect. It is concluded that the vitamin E inactivation process had an effect on the lipids present in the food treated. It is altogether probable that some fat-fraction, yet unidentified, was oxidized by the treatment. Since the chief concern of this study is the factors which influence gene expression, it does not matter which food component is actually affected by the inactivation process.

TABLE 2

PROLONGATION OF LARVAL AND PUPAL STAGES OF DEVELOPMENT DUE TO VITAMIN E INACTIVATION AND A CONSEQUENT INCREASE IN FACET NUMBER IN AN ULTRABAR STRAIN

		I Control	II Experimental organisms cultured on a medium containing 1 per cent. ferric chloride by weight	III Experimental organisms cultured on a medium treated for Vitamin E inactivation
Mean larval period in hours		167	178	272.50
Mean pupal period in hours		120	131	154.00
Facet number	Males	29.27	29.88	31.97
	Females	26.00	27.19	29.27
Standard deviation of the mean	Males	±4.20	±2.58	±2.76
	Females	±2.42	±2.42	±3.36
Standard error of the mean	Males	±.66	±.40	±.46
	Females	±.46	±.46	±.67
Statistical significance	Males	− with II	+ with III	+ with I
	Females	− with II	+ with III	+ with I

(+ = statistical significance, − = absence of significance.)

The absence of thiamin and the inactivation process of vitamin E had no appreciable effect on the mortality rate, fertility or sex ratios, but they did slow up the physiological and metabolic activities which led to normal development. This slowing up with a consequent prolongation of development had a peculiar effect on the expression of the factors responsible for facet number. The expression of these gene-differences at the bar locus was

decreased rather than enhanced. Braun (1939) and Green and Oliver (1943) postulated an enhancement of gene expression for the characters studied when the developmental time of the larval stage was prolonged. This enhancement of expression was reflected in a more pronounced effect on the end-characters than when development was normal.

The conclusions of Braun are based on the hypothesis that the notched-wing character he studied was due to a resorption by substances elaborated by the gene for the character. The longer the time of development, the longer the time the gene has to act and hence the greater the amount of resorptive substance elaborated. Green and Oliver suggest that from the data they have obtained it would seem that the gene for vestigial wing belongs in the category of antimorphic genes. This would mean that the gene for vestigial acts to prevent normal differentiation of wings. Prolongation of development permits an increase in the time of gene action so that in the case of heterozygous *vg* sufficient substance to cause scalloping is produced. The quantity of vestigial substance elaborated depends upon the dosage of *vg*, the allele present and the extent of prolongation of the third larval instar.

It is evident from the results of the present study that the gene differences at the bar locus do not express themselves in a manner comparable to the factors studied by the above-mentioned authors. In the case of eye development, a study of the embryology has shown that there is no evidence that resorption takes place at any time (Steinberg, 1941). Also, from the data obtained it is not possible to associate the mode of gene expression for facet number with that for vestigial. In the latter case the prolongation of development enhanced gene expression, while in the former case it was decreased. If the gene differences for facet number at the bar locus were enhanced in their expression, then a greater reduction in facet number would result, but in the present study we have found that the facet number was increased by the prolonged developmental time.

It can be postulated that the factors for facet number are slowed in their expression by prolongation of the larval period which results in an increased number of facets. This postulation is bolstered by the investigations of Luce (1939, 1940 and 1942) as well as by the results of numerous investigations on temperature effects on *Drosophila melanogaster*.

Summary

A diet deficient in thiamin hydrochloride greatly prolongs the larva-pupa time of development with a consequent effect on facet number in a bar strain of *Drosophila melanogaster*.

A culture medium treated for inactivation of vitamin E by use of a mixture of ferric chloride and ether not only has the vitamin E inactivated but some other food component which is thought to be a fat-fraction. This treated medium considerably prolongs larval-pupal development with a consequent effect on facet number.

The facet number of flies cultured on both types of culture media was increased significantly. This would indicate a decrease of gene expression due to the prolongation of development caused by the dietary deficiencies.

From these data it can be concluded that the factor responsible for facet number in *Drosophila melanogaster* does not function in a manner comparable to the so-called antimorphic gene *vg* which was studied by Green and Oliver (1943) nor the gene for notched-wing which elaborates resorptive substances as postulated by Braun (1939).

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

In these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Tempo and Mode in Evolution. By GEORGE GAYLORD SIMPSON. New York: Columbia University Press, 1944: i–xviii, 1–237, figs. 1–36. $3.50.

THIS newest first-rank treatise on evolution may be characterized as brilliant, but not flashy; brimful of fresh ideas for the researcher, but hardly fitting the needs of an elementary student; comprehensive, though making no pretense at covering the whole field of organic evolution. It is neither a textbook nor a compendium—several of each have recently appeared—but rather the vehicle by which the gifted author presents the philosophical conclusions, regarding the rates and methods of evolution, which he has reached during the course of his extensive researches and deliberations in paleontology. Though the background and base data are largely those of a paleontologist, a broad and objective viewpoint has been sought and rather consistently attained. Usually with success and wisdom the fossil record is continuously viewed in the light of the facts and interpretations of modern genetics, as well as from the eyes of a paleontologist. As a result Simpson, unlike many of his colleagues, quickly sets aside all Lamarckian principles, dispenses with metaphysical concepts of inner drives and strivings for perfection, and merely annotates as inacceptable to him such common ideas as those of "exuberant racial youth" and of "racial old age." He treats as overgeneralizations the dicta of some paleozoologists that all evolution is adaptive, that all evolution has definite trend and that evolution is never reversible, although

it is clear that he regards these general principles as having usual validity.

As one would expect of a coauthor of the sound and thorough treatise, Quantitative Zoology, Simpson repeatedly quantifies data on the tempo and mode of evolution —judiciously and with due reservations. Some approximations thus reached are very interesting and suggestive. For example, it is estimated that gene mutations may be expected to occur simultaneously in an individual only once during a time period four times the age of the earth! (Perhaps Goldschmidt will take this as evidence either of the evolutionary inadequacy of gene mutation or of the non-existence of genes.)

Though he avers that "the materials for evolution and the factors inducing it are believed to be the same at all levels," and therefore joins many other biologists in attacking Goldschmidt's sharp distinction of microevolution (within species) and macroevolution (between "good" species), Simpson in passing uses not only "micro-evolution" and "macro-evolution" but further proposes a new etymological hybrid, "mega-evolution," to refer to the large-scale phylogeny of major groups. He explains that most paleontologists regard such major evolution as more distinct from macroevolution as treated by Goldschmidt, than macroevolution is from microevolution. In a way he outdoes Goldschmidt, for he has not only expanded on that geneticist's classification but has also presented a three-fold break-down of evolution on each of two additional bases. In the second classification, with an apology for the introduction of the new terms, he distinguishes rates and hence kinds of evolution (and the phyletic lines involved) as *bradytelic* (low-rate, as in "arrested evolution"), *horotelic* ("standard-rate," whatever that may be) and *tachytelic* (high rate). As a third classification, referring to modes, evolution is typed into (1) *speciation,* characteristic of low taxonomic levels; (2) *phyletic evolution,* typical of groups from genera to families but not strictly confined to these ranks;

and (3) *quantum evolution*, principally exemplified in the higher taxonomic categories, and therefore roughly corresponding with "mega-evolution," though the modified Goldschmidtian classification seems to have been forgotten in the final arrangement. Many biologists will see no fundamental qualitative difference between bradytelic and horotelic rates or lines and will fail to appreciate the need for such terms. "Speciation" has some distinctive features and will surely remain in use, largely because it is the aspect of evolution that is subject to genetic research and that is at present the evolutionary field of chief concern to most taxonomists. "Phyletic evolution," emphasizing consistent trends, and "quantum evolution," focused on the diversified early development of a group or on the rapid transition from one "adaptive peak" to another, are useful points of view, but seem to me to apply almost equally to all taxonomic levels and to intergrade completely. I would regard them as stages in phylogeny, "quantum" preceding "phyletic," rather than as different sorts of evolution.

Lest it appear that I disagree widely with Simpson I have compiled and present below with enthusiastic approval a series of his statements, selected to represent his evolutionary philosophy (sentences and phrases have been rearranged, with some deletions and with some conjunctions added):

> The extent and nature of variability are themselves important group characters subject to natural selection and other evolutionary factors. Segregation or selection of intragroup [intraspecific] variability can give rise to new groups at a potentially rapid evolutionary rate. Evolution on the basis of existing variability cannot proceed beyond about the specific level. In continuous phyletic evolution of large populations at moderate rates variability tends toward a relatively constant level. Progressive loss of variability does not appear to be a usual or general principle of evolution; loss of hereditary variation is balanced by the appearance of new mutations. Rate of sustained evolution normally shows little or no correlation with variability, but conservative groups are sometimes exceptionally variable. Extreme variability is more likely to appear in degenerating than in rapidly progressing structures.
>
> Mutations with extremely small phenotypic effects that occur at moderate or low rates, such as have repeatedly been found in experimentation, are consistent with [observed] rates of evolution; their tempo and mode are not

necessarily or primarily controlled by mutation rates. Paleontological evidence does not require saltation.

The appearance of a mutant individual is not evolution. Only populations, not individuals, evolve. However profound or systemic a mutation may be, it is difficult to see how its eventual effect on populations can be independent of the neo-Darwinian factors. The sudden appearance of a really new individual reaction system morphologically of generic or higher grade would not produce a new genus or higher genetic group unless and until this system came to characterize an isolated population.

Many phyletic lines progress in a regular manner toward a condition that has appeared to some students so fixed as to be predestined. "Orthogenetic" evolution, however, is almost invariably adaptive. Observed rectilinear sequences are usually most consistent with the theory that orthoselection is the dominant or primary factor, and some are not logically explicable in any other way. There are several ways in which momentous effects can arise within the scope of the neo-Darwinian theories of mutation–selection. There is no good evidence that a trend has ever been continued by momentum beyond a point of advantageous or selectively neutral modification or has ever been the direct cause of extinction. The most general explanation is simply that a phylum has become highly specialized and adaptively specialized and a change in selective influences has made the specialization secondarily inadaptive: lag, but not momentum.

Selection is a truly creative force. It is one of the crucial determinants of evolution. New groups invariably represent a major ecological adaptive change. Characters hitherto supposed to be nonadaptive are quite definitely adaptive and characters of unknown adaptive value are nevertheless definitely selected by differential survival. In large populations evolution is extremely slow except under the influence of selection, and then it is almost purely adaptive in type and approximately proportional to selection intensity in rate.

On the combined basis of paleontological and relative-growth data "four basic theorems concerning rates of evolution" are thus stated:

1. The rate of evolution of one character may be a function of another character and not genetically separable even though the rates are not equal.
2. The rate of evolution of any character or combination of characters may change markedly at any time in phyletic evolution, even though the direction of evolution remains the same.
3. The rates of evolution of two or more characters within a phylum may change independently.
4. Two phyla of common ancestry may become differentiated by differences in rates of evolution of different characters, without any marked qualitative differences or differences in direction of evolution.

Some other principles of phylogeny are stated by Simpson in the following words:

The usual sequence is for one dominant group to die out, leaving the zone empty, before another group becomes abundant. For most animals the ex-

panding phase is shorter than the contracting phase so that groups narrowly distributed are more likely to be relicts than new or expanding groups, but relatively restricted area characterizes both the earliest and the latest stages of phyletic history. The best part of the paleontological record is made up of lines that evolve in approximately one direction over long periods of time. It is, however, obvious that rectilinear evolution is far from universal. More specialized branch phyla tend to evolve more rapidly than their parent stocks and more specialized phyla tend to become extinct before less specialized: the rule of the survival of the relatively unspecialized. Phyla evolving more slowly or not evolving except for small and apparently random variations sometimes continue indefinitely, even for hundreds of millions of years, in this evolutionary state of rest.

In view of the fact that the book was completed and put through the press while the author was in military service it seems gratuitous to point out what appear to be minor defects. It is to be hoped, however, that the next edition will carry a summary of the author's views, and will give greater attention to such pertinent zoological data as explosive evolution in unsaturated Recent habitats. The chapter on Organism and Environment—the least satisfying to the reviewer—might be revised. Some details of terminology and of organization might be reconsidered, though as a whole, the text is lucid and remarkably free of errors. What fellow workers will most want in the next edition or in a supplementary treatise are more facts and interpretations from the author's great store and some consideration of evolutionary problems that are given little or no emphasis in the present volume.

The Dice of Destiny. An Introduction to Human Heredity and Racial Variations. By DAVID C. RIFE. Columbus, Ohio: Long's College Book Co., 1945: 1–163, figs. 1–23. $1.75.

AFTER being surfeited with rebuttals of the Nazi philosophy of racial superiority, all based either on the almost equally unscientific idea that human races do not differ at all in mental characters or on the still more absurd notion that races do not exist, we find real relief in the appearance of a scientifically sound treatise on human race problems. The human biologist shows himself able to avoid the pseudo-science that seems to pervade in part the thought of many physical anthropologists as well as sociologists.

After introducing his little book as a treatment on politico-genetics, as contrasted with medical genetics, Rife proceeds to demonstrate, in words that may be understood by those untrained in genetics or any other phase of biology, how individuals differ genetically in "a matter of taste" (to phenyl-thio-carbamide), in blood antigens, in hair, eye and skin color, in various other physical features, in dermatoglyphics, in handedness, in mental capacity, in special abilities; further, how racial groups are characterized by different frequencies of the genes responsible for the physical and mental make-up of the individual. It is shown that other characters are not consistently correlated with Nordic blondness, that Jews are superior in some respects, that no race has a monopoly of desirable characters, that racial differences are quantitative not qualitative.

Interspersed with this line of presentation are simplified discussions of human heredity, devoid of any cytogenetic detail, and of sex ratios and of traits associated with sex. There is also a biologically sound treatment of the interplay between heredity and environment, each of vital and indispensable importance in determining the characteristics of individuals and hence of racial and national groups.

Toward the end of the book the conclusions of human biology are increasingly linked with ethics to build up a democratic philosophy of individual and racial conduct. Though in such discussions the author may have transgressed the limits of science he has not overstepped our current ideals of human relationships.

Dice of Destiny is a book of great potential value, an antidote to the poisons of Nazi philosophy and to the unfounded rebuttals that have flooded the public. It should pass through several editions, which, incidentally, would provide means for correcting such minor defects as typographical errors and occasional passages that are not clear.

The Distribution of the Birds of California. By JOSEPH GRINNELL AND ALDEN H. MILLER. Pacific Coast Avifauna (Cooper Ornithological Club, Berkeley, Calif.), 27, 1944: 1–608, frontisp., figs. 1–57. $6.00 (unbound), $7.00 (bound).

INFINITE precision has been the obvious watchword in the preparation of this large volume on the distribution of the birds of California. For each of the 644 kinds of birds verified as of natural occurrence within the state limits there is given, for California, the reproductive and migratory status, with an indication of abundance; the geographic range in elaborate detail, supported by a great wealth of references; and the habitat. For the commoner species there are stated the habitat features that appear to be of significance in determining occurrence and distribution. Systematic discussions are limited to notes explaining the status that is accepted by the authors. Blind adherence to the American Ornithologists' Union Check-list is avoided and condemned. I note also a criticism of the "freely exercised vogue for joining in one species related geographically complementary forms."

Of particular value are the 57 excellent maps showing, for the breeding season, the record stations and distribution of each subspecies within California, for the species (with a few exceptions) having two or more named races in the state. It is recognized that the maps as prepared fail to delimit areas of intergradation and conceal the discontinuities within ranges. Failure to identify and to spot map intergrades I regard as a major defect not only of this treatise but also of ornithological work in general, and makes one wonder how far "authority" still rules in the science of birds.

The treatment is rigidly confined to the state of California. Small insert maps showing the entire distributional limits of the forms breeding in California would have greatly increased the value and utility of the book.

This source book, of infinite detail, will be indispensable to all students of the birds of western North America, and

will be valuable also to workers in ecology, zoogeography, game management and speciation. Conspicuously absent, however, is any synthesis of the data: no maps of life zones, communities or faunal districts of the state; no principles regarding zoogeographical patterns, differentiation centers, barriers, size of ranges, types of intergradation, etc.; no generalizations on the ecological and habitudinal control of distribution. Naturalists in many fields will eagerly await the fulfillment of the promise that such syntheses will be presented in a separate work.

NOTICE OF NEW BOOK

Amphibians and Reptiles of the Chicago Area. By CLIFFORD H. POPE. Chicago: Chicago Natural History Museum, 1944: 1–275, pls. 1–12 (6 in color), figs. 1–50. $1.75.—Like many another youth Clifford Pope idolized the late Raymond Ditmars, read and reread his works on reptiles, but unlike the rest, Pope grew up to replace his former mentor as America's foremost author on popular herpetology. He has the uncommon ability to present scientific material in an informative, fascinating, and unstilted style without sacrifice of authenticity.

It is gratifying to find that emphasis is placed on a general life-history account of each species, including habits, reproduction, growth, age, food, enemies, importance, habitat, occurrence, and maintenance in captivity. Keys and recognition characters are adequate, but identification is treated as a tool, not an end. Each of the 52 species is well illustrated, 16 in color, and references are appended.

Although prepared primarily for residents of the Chicago area, this work will fill a general need, especially in the North Central states region. Readers of general interests will find in it a great fund of well-organized, reliable information. Every herpetologist will want it on his desk.—REEVE M. BAILEY, *Museum of Zoology, University of Michigan, Ann Arbor, Mich.*

SHORTER ARTICLES AND DISCUSSION

A MORE MEANINGFUL FORM OF THE "LOGISTIC" EQUATION

EQUATIONS of growth are legion; even those which are called "logistic" occur in a great variety of forms (see, for instance, Kavanagh and Richards, 1934; and Wilson and Puffer, 1933). Of these, perhaps the most attractive to the rational biologist is:

$$N = \frac{K}{1 + e^{a-bt}} \tag{1}$$

which results from the integration of:

$$\frac{dN}{dt} = bN\,\frac{K - N}{K} \tag{2}$$

where:

N = number of organisms (or any similar measure of growth or multiplication)

K = upper asymptote of N

b = "biotic potential" or potential rate of increase in the absence of environmental resistance

t = time

e = base of logarithms

The above differential equation, first applied (in somewhat different form) to the problem of biological growth by Robertson, 1908, and by Ostwald, 1908, owes its attractiveness to the fact that it includes only terms which refer to identifiable entities, combined in accord with assumptions which seem reasonable to the experimental biologist. The rationale behind the equations has been clearly expounded by Gause, 1934, whose symbols I use. It will be noted, however, that during the course of integration the expression picks up the arbitrary constant a. To those who hold with Jacques Loeb (see Osterhout, 1928) that "all the observed phenomena [of biology] should be expressed in the form of equations containing no arbitrary constants," this constant, a, constitutes a blemish in the logistic function. From such a point of view it is desirable to eliminate the offending member. This can be done as follows:

Consider the value of the function when $t = 0$, i.e., when $N = N_0$.

$$N_0 = \frac{K}{1 + e^{a}} \tag{3}$$

$$e^a = \frac{K}{N_0} - 1 = \frac{K - N_0}{N_0} \tag{4}$$

$$a = \ln \frac{K - N_0}{N_0} \tag{5}$$

Having evaluated a we may write:

$$N = \frac{K}{1 + e^{\ln \left(\frac{K - N_0}{N_0}\right) - bt}} \tag{6}$$

which, by the definition of logarithms, is:

$$N = \frac{K}{1 + \left(\frac{K - N_0}{N_0}\right) e^{-bt}} \tag{7}$$

Thus the arbitrary constant is replaced by an expression involving only the asymptote of the growth function (K) and the initial value (N_0). That a can be evaluated was recognized by Gause in his treatment of graphical methods (p. 149); but he nevertheless retained the less meaningful constant in his logistic equation. Undoubtedly others who have actually worked with logistic equations have also realized the significance of a, but little or no overt notice appears to have been taken of the situation.

Whether this substitution will be convenient or not will depend on the nature of the problem attacked. In the case of natural populations, the value of N_0 needed for a "good fit" may sometimes be obviously false, due to the gross accidents of fortune that attend the establishment of new enterprises. In that case one might prefer to retain the more cryptic symbol a in the exponent; or, one might use another common form of the equation:

$$N = \frac{K}{1 + Ce^{-bt}} \tag{8}$$

where: $\ln C = a$.

With experimental material, however, which permits the direct determination of N_0, equation (7) should be preferred. In fact, the possibility of employing this more meaningful form of the growth equation may be regarded as a test of the validity of the logistic "law" and of the degree of the investigator's control of the experimental situation.

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INDUCTION OF OVOTESTIS IN ESTROGEN TREATED ADULT MALE TELEOST, XIPHOPHORUS HELLERI

In a study on sex-differentiation in the teleost, *Xiphophorus helleri*, Essenberg (1923, 1926) found that 50 per cent. of the young females transformed to males before sexual maturity was reached and two cases of natural complete sex-reversal were observed in parturient females. He concluded that any agent or condition which tends to decrease the capacity for female sex hormone secretion beyond a certain limit becomes an immediate factor in sex-reversal in the female of this species. This postulation was substantiated by the administration of testosterone propionate, which caused not only the morphological masculinization but accompanied by developing of a male gonad with spermatogenesis in approximately 50 per cent. of the experimentally treated *Xiphophorus* females (Baldwin and Goldin, 1939, 1941). The appearance of changes in secondary sex characters of the female *Xiphophorus*, although no clear testicular differentiation was noted in the ovary, by treatments of androgens has also been reported (Witschi and Crown, 1937; Regnier, 1938). Moreover, Witschi (1942) mentioned the unpublished results of his student Crown (1941) that many *Xiphophorus* females became masculinized by treatment of estrone; other cases of complete sex-reversal were observed in these females when treated with gonadotrophin from pregnancy urine (Baldwin and Li, 1942).

While the sexuality of female *Xiphophorus helleri* appears to be unstable and changeable, detailed investigation on the actions of estrogenic hormones on the male fish of this species is highly important before one can come to a solution regarding the sexual development of the fish. Regnier (1938) reported that injections of estrogen into *Xiphophorus helleri* and *Lebistes reticulatus* sup-

pressed the male secondary sex characters and inhibited the spermatogenesis, but the formation of ovocytes in the testes has not yet been reported. However, Berkowitz (1938, 1941) has shown that estrogen feeding induced a mosaic of male-female characters in the male *Lebistes reticulatus* and produced a marked inhibition of the testis together with its transformation into an ovotestis, and almost complete reversal of testis to ovary was observed, whereas injection of estrogens caused less reversal and egg cells rarely appeared. We have continued to study the influence of estrogen in mature male *Xiphophorus* to determine if possible to what extent this form can respond in a comparable way to the results reported to occur in other species.

Experimental. Two series of adult male fish, besides the control and other experimental groups, were injected retroperitoneally with alpha-estradiol benzoate (Progynon-B) :[1] one series of 25 fish each received a weekly dosage of 0.03 mg; and the other series of 37 fish each received the same amount of estrogen at intervals of 15 days. The experiment was conducted over a period of 18 months, and the treated animals were sacrificed for histological observations after varying lengths of administrations. In general, there were no estrogenic effects on the already well-developed gonopodium and sword of the caudal fin, although perhaps there was a slight fading in general bodily coloration. The testes showed different degrees in hastening of spermatogenesis, atypical release of spermatozoa, hypertrophy of interstitial tissue, atrophy of sperm ducts, tubules and the gonad as a whole, and other pathological conditions. A detailed study of these responses will be reported elsewhere. The present communication notes two cases of ovotestis which were induced in the second series of treated animals that after the treatment had been discontinued for some time.

The first ovotestis was observed in a fish (MPB 41) which had received 12 injections and was killed 4 months after the last injection. The hypertrophied sperm ducts were distended with a large number of spermatophores and formed the main bulk of the exhausted gonad. The thin cortical portion consisted of sperm tubules, which appeared destitute of germ cells, and scattered cysts of gametogonial cells; neither spermatocytes nor spermatids were noted. The epithelium of these ducts and tubules were more

[1] The authors wish to thank Dr. Max Gilbert, of the Schering Corporation, for the Progynon-B.

or less atrophied, and there were increasing amounts of interstitial tissue. Three small ova were found in the middle part of the elongated gonad. One oocyte, measured 80 μ by 65 μ in diameter, was located between the fused edges of the two gonadal lobes (Fig. 1). The other two, measured 40 μ by 52 μ and 65 μ by 78 μ in diameter respectively, were clustered beneath the external epithelium of the gonad. The vesicular nuclei of these oocytes contained clearly defined nucleoli, but no yolk granules were formed in the cytoplasm. Some cysts of large gonial cells were always associated with these egg cells.

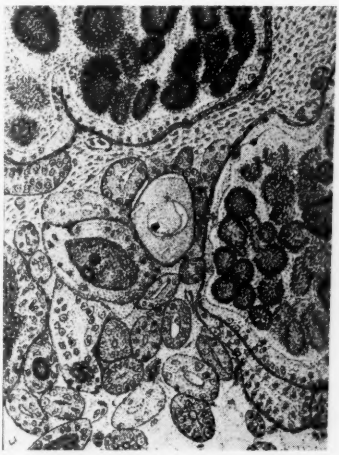

FIG. 1. Section of part of an ovotestis (MPB 41) showing an oocyte among the atrophied sperm ducts and tubules. ×220.

Another ovotestis was found in a fish (MPB 71) in which treatment had been discontinued for 6 months after it had received 24 regular injections. Histological examination (Fig. 2) revealed that the gonad resembled an immature testis with large cysts of various stages of spermatogenic cells except the mature sperm. The small centrally placed gonoducts were devoid of any germ cells and no spermatophores were found. The sperm tubules were extremely rare. At least 12 small ova were counted in the central part of this gonad and many of them showed a certain degree of disintegration in their nuclear structures. The diameters of these egg cells were reduced, ranging from 105 μ by 78 μ to 65 μ by 40 μ or even smaller. The follicular epithelium was

also disorganized, hence, most of these ova were merged indistinctly into the surrounding spermatogenic cells. Other cell structures were similar to those as described in the previous case. The scarcity of interstitial tissue was very conspicuous in this gonad. It seemed that the rejuvenized testicular tissue would eventually overrun the degenerating ova.

Discussion. Our results show that there is a capacity for the development of ovotestis in the mature male *Xiphophorus helleri* when the animals had undergone prolonged treatments of small doses of estrogens. Continuous administration of high doses of

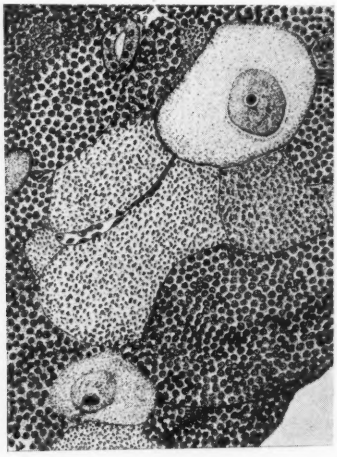

FIG. 2. Section of part of a second ovotestis (MPB 71) showing oocytes and cysts of spermatogenic cells. ×308.

estrogens, however, were not effective in developing egg cells, because they caused a marked suppression in the development of germ cells and the atrophy of testicle. These effects are somewhat similar to those found in the estrogen treated male *Lebistes reticulatus* (Berkowitz, 1941). The gametogonia which lie upon the periphery of the ovotestis appear to be the immediate source in the formation of ova. It is interesting to note that there is no instance of reversal of the testis in adult male amphibians either naturally or by experimental procedures as the "male cortex" disappears in older males so that they lose their amphisexual character of the larval gonad (Humphrey, 1942).

Essenberg (1926) claims that in *Xiphophorus helleri* sex is

determined and controlled by the sex hormones derived from the ovary and testes; chromosomes are not considered as having the function of sex determination and control. Ralston (1934) notes 24 tetrads in the first maturation division of this species and suggests that one of the large chromosomes which takes its position on the equatorial plate slower than the others, and advances quickly its precocious divided daughter components to the poles of the spindle, represents a sex chromosome. He interprets the condition as one in which the male is homogametic and infers the sex chromosomes of *Xiphophorus* as the ZZ–ZW type. In a similar cytological study, however, Friedman and Gordon (1934) have not confirmed the differentiation of sex chromosomes in this species. Unfortunately, sex-linked factors have not been discovered in *Xiphophorus helleri*. Thus far the genetic investigations upon this species have not yielded conclusive results concerning the type of sex-determining mechanism. From the cross-breeding experiments between *Xiphophorus helleri* and *Platypoecilus maculatus*, which is known as ZZ–ZW type, Kosswig (1935) concludes that the sex of *Xiphophorus* is determined phenotypically by environmental influences. Goldschmidt (1937) reinterprets Kosswig's results, assuming somewhat a ZZ–ZW type ($FFMM_1 = ♀$; $FFMM = ♂$) in line with the work done upon the undifferentiated races of frogs by Witschi. Whatever the hereditary mechansim for sex determination may be, or even without any such mechanism as indicated by Witschi (1939) and others, the tendency for maleness is dominating in *Xiphophorus helleri;* this is supported not only by the facts in their sexual development but also by the experimental administrations of sex hormones. There is no complete sex reversal from male into female observed in the adult male of this species and the ovotestis which is induced by estrogen treatments may gradually change back to a testis when the hormone is withdrawn.

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THE EFFECTS OF LOW INCUBATION TEMPERATURES ON THE EXPRESSION OF ROSE AND DUPLEX COMB, CREST, MUFFS, FRIZZLED, NAKED NECK AND TRIPLE SPURS IN THE DOMESTIC FOWL

STURKIE (1943) demonstrated that polydactyly in the fowl could be suppressed by lowering the temperature of incubation at certain stages of development. Most of the treatments were employed upon embryos from 2 to 5 days of age, and, in most cases, before differentiation of the character. Warren (1944) also suppressed polydactyly with low temperatures, but not to the extent as that reported by Sturkie. The treatments which were effective in suppressing polydactyly, and others, were used in an attempt to modify the expression of rose and duplex comb, crest, muffs, frizzled, naked neck and triple spurs. All these characters are unifactorial-dominant ones.

The stages of development at which the low temperatures were applied varied, but an attempt was made to employ the treatments at what was considered the probable critical period for the characters; however, this was not the case for a number of treatments,

particularly those employed at two, three and five days on crest, duplex combs and muffs, since these treatments were designed and used mainly for the effect on polydactyly.

A Houdan male, homozygous for crest, duplex comb and muffs, was mated to normal White Leghorn females. Likewise a homozygous frizzled male was mated to non-frizzled females. Thus

TABLE 1

THE EFFECTS OF LOW TEMPERATURE UPON THE EXPRESSION OF ROSE AND DUPLEX COMB, CREST, MUFFS, FRIZZLED, NAKED-NECK AND TRIPLE SPURS

Age of embryo treated Days	Treatment	Character and no. classified	
Two	16 –48–R[1] 3.3°–34–C	Crest, Duplex comb, Muffs	94*
	124 – R 34°– C	Rose Comb 6 : Naked Neck Single Comb 12 : Normal	13 8
	12 –96–R 10°–34–C	Rose Comb 5 : Naked Neck Single Comb 5 : Normal	14 17
Three	16 –48–R 34°–34–C	Crest, Duplex Comb, Muffs	79
	96 – R 34°– C	Triple Spur Normal	7 3
Five	12 –96–R 3.3°–34–C	Crest, Duplex Comb, Muffs	11
	96 – R 34°– C	Triple Spur Normal	10 7
Six	96 – R 34°– C	Triple Spur Normal	6 6
	12 –60–R 3.3°–34–C	Crest, Duplex Comb, Muffs	17
Seven	12 –72–R 3.3°–34–C	Crest, Duplex Comb, Muffs	14
Eight	12 –72–R 3.3°–34–C	Crest, Duplex Comb, Muffs	9
Nine	16 – R 3.3°– C	Rose Comb 20 : Naked Neck Single 20 : Normal	23 18
Ten	16 –72–R 3.3°–34–C	Frizzled Non-Frizzled	74 4

* Progeny showing contrasting characters were not obtained.
[1] 16 hours at 3.3° C. then 48 hours at 34° C. and the remainder of incubation period at control temperature (37.5° C.).

all the embryos or progenies of these matings under control conditions were expected to exhibit, and did show the dominant characters, except in the case of the frizzled mating where a few normal or questionable offspring were obtained. The rose-comb, triple-spur and naked-neck males which were mated to normal females were heterozygotes and hence a 1 : 1 ratio was expected and obtained in their progeny under control conditions.

The results of the treatments upon the expression of these characters are shown in Table 1. It is obvious that these treat-

ments did not affect the expression of any of the characters except possibly the treatment on rose comb at two days. In view of the results of treatments at three and nine days on this character, it is not believed that the treatment at two days accounts for the shortage of rose-combed progeny thus obtained. The percentage of non-frizzled progeny obtained after treatment was no greater than that secured under control conditions, and these may have resulted from the action of a modifying gene or genes for frizzling such as reported by Landauer (1933) and Hutt (1936), or from an error in classification.

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